Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

A41.7
R 313AR
Copy. 2

ARS 91-101
July 1971

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS' SALMONELLOSIS WORKSHOP

Held At

Las Vegas, Nevada

June 22, 1970

Agricultural Research Service

U. S. DEPARTMENT OF AGRICULTURE

Views expressed by the speakers and reported here are not necessarily those of the U.S. Department of Agriculture.

Italicized numbers in parentheses refer to references at the end of each paper. References, figures, and tables are reproduced as they were supplied by the writer of each paper. .

Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

Prepared in
Animal Health Division
Agricultural Research Service
U.S. Department of Agriculture
Hyattsville, Md. 20782

PREFACE

Because of the continuing importance of avian salmonellosis to all phases of poultry and egg production and to public health, the Salmonellosis Committee of the American Association of Avian Pathologists (AAAP) sponsored a 1-day open workshop on June 22, 1970, prior to the American Veterinary Medical Association (AVMA) convention in Las Vegas, Nev. Scientists in the field of avian salmonellosis presented short resumes of their work, followed by informal discussion. Over 60 representatives of the poultry industry, regulatory agencies, and State and Federal research institutions were in attendance.

The discussions and exchange of ideas that took place opened many avenues for the development of more successful control programs for salmonella infections in poultry. This proceedings is the record of the workshop. It includes summaries of the reports presented and other information developed during the discussion sessions.

CONTENTS

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS' SALMONELLOSIS WORKSHOP

Held at Las Vegas, Nevada, June 22, 1970

THE SCOPE OF THE AVIAN SALMONELLOSIS PROBLEM

Birch L. McMurray[1]

The purpose of this brief presentation is to discuss the broad aspects of salmonellosis as it pertains to the poultry industry. A look at the list of topics to be discussed makes one realize the scope of this problem. Consequently, I will in no way attempt to go into detail of each of these areas.

The goal of all of us here today is to explore the various ramifications of salmonellosis as it pertains to the poultry industry, to bring this information to the industry through publication of *Avian Diseases*, and to update recommendations for the prevention and control of avian salmonellosis.

I view the broad field of salmonellosis as being divided into two major categories. These are:

1. Clinical salmonellosis of the poultry industry, and
2. Salmonellosis of public health concern.

Since our committee is concerned with salmonella other than pullorum disease and typhoid, we can readily say that, directly or indirectly, our major concern comes because of the public health aspect of salmonellosis.

Clinical salmonellosis and arizona infections do occur, and in individual flocks of chicks and poults either of these disease conditions may result in considerable losses. Yet, neither of these diseases constitutes one of the major diagnoses of disease diagnostic laboratories. In 1968, a total of 1,532 cases of salmonellosis out of a total of 78,981 accessions was reported by the Southern, North Central, and Northeastern Conferences of Avian Diseases. Also, 144 cases of arizona infections were reported. This is less than 2 percent (*3*).

Salmonellosis may be a problem for both breeder flocks and hatcheries, but, with the exception of pullorum disease and typhoid, the industry has no reliable and practical way to monitor for serotypes other than *Salmonella typhimurium*.

Salmonellosis cases in people are rarely if ever traced back to grade A table eggs, but, when egg related, are caused mostly by undergrade eggs that have been incorrectly processed or improperly cooked *(2)*.

Arizona infections are more of a clinical problem than salmonellosis in turkeys; however, clinical salmonellosis is more prevalent in turkeys than it is in chicks.

The presence of salmonella organisms on dressed broilers and turkeys constitute a potential public health hazard.

The presence of salmonella organisms in rendered animal, poultry, and marine products and, in turn, in livestock and poultry feeds has and continues to pose a major problem to both the rendering industry and the feed industry. The USDA, through the cooperative State-Federal Salmonella program, has very active work underway in this area. Their work shows 56 percent of marine rendering plants and 31 percent of animal and poultry rendering plants produced free products in 1969 (*7*).

The same serotypes found in rendered products have a high degree of correlation with those serotypes found in feeds. Yet, the serotypes found in feeds do not have a high degree of correlation with those serotypes found in poultry. An example here would be that *Salmonella eimsbuettel* is frequently found in rendered products and in feeds, but is considerably less frequently found from poultry. Likewise, *S. typhimurium* is seldom found from either rendered products or feeds, but is very commonly found from poultry and other animals. According to the Salmonella Surveillance Report of the NCDC, there is about a 50 percent correlation among the 10 most prevalent serotypes among human and nonhuman sources *(6)*.

[1] Manager, Veterinary Research Central Soya Company, Inc., Decatur, Ind.

The NCDC report shows that salmonellosis ranks third as the cause of food-borne diseases–13.3 percent of the food-borne outbreaks were traced to salmonellae during 1969, while 25.3 percent were traced to staphylococcus, and 17.5 percent to *Clostridium perfringes*. In numbers of people involved, salmonellosis also ranked third, with 6.6 percent of the total number of people involved. Poultry meat, with turkey meat being the greatest offender, led other food products as the cause of salmonellosis in people *(5)*.

However, it is estimated that about 2 million cases of salmonella in people per year occur with a loss of no less than $300 million to the American economy *(1)*.

Contrary to this view is that expressed in *Feedstuffs* by R. Dean Thomas who states, "that salmonellosis ranks rather low as a disease of importance when compared to other disease problems. In checking with the management of some of the larger companies, it was learned that diarrhea and/or food poisoning from all causes, not just salmonella, would be incidental compared to the time loss from colds, flu, female problems, etc." He questions whether or not the attention being given to salmonella is warranted. He states that 22 governmental agencies in the World Health Organization are participating in the study of salmonellosis. In relation to major Federal programs that are being "phased out" because of a lack of money in both the human and livestock areas, he questions whether or not we can afford to continue to work on the salmonella problem *(4)*.

During the past year, the National Academy of Sciences has published their report: *An Evaluation of the Salmonella Problem.* This report, of course, looks at the broad aspect of the salmonellosis problem. Its recommendations include coverage of the following aspects:

1. Contamination of raw animal products and drinking water.
2. Contamination of processed foods, feeds, and drugs.
3. Mishandling of food during preparation and serving.
4. Education and training.
5. Research.
6. Other.
7. Implementation *(1)*.

Regardless of the diversity of opinion and the confusing and unanswered questions, the fact is that the problem of salmonellosis is here. All segments of the poultry industry are involved. No doubt many of you feel that our duties should end with the area of feed manufacturing and the production of eggs, chicks, poults, or ready-to-market broilers and turkeys. However, the ultimate product that our industry produces today is either processed and ready to be prepared or is already prepared for the American public's table. Consequently, our duties do not end with the successful production of a bird or an egg. Already, regulations are being considered for bacteriological control of processing areas and of finished poultry food products. It is my opinion that our area of concern should encompass the final product as well as the breeder, the hatchery, the chicken, and the turkey, because actually the area of clinical salmonellosis is minor compared to the public health aspect.

Salmonellosis will not disappear by being ignored. It needs thought and work and not complacency. It is our job here today to devote our time and thinking to evaluating the poultry industry's problem and to make recommendations that apply to our industry.

LITERATURE CITED

(1) Committee on Salmonella, Division of Biology and Agriculture, National Research Council. A Report of the U.S. Department of Agriculture and The Food and Drug Administration, U.S. Department of Health, Education, and Welfare. Natl. Acad. Sci. Pub. 1683. Washington, D.C. 1969.

(2) Committee on Salmonella, Poultry and Egg National Board, National Communicable Disease Center. Committee Report, January 1969. Atlanta, Ga.

(3) Southern Conference, North Central Conference, and Northeastern Conference, Annual Disease Reports. Avian Dis. vol. 13, No. 4, pp. 897-931. November 1969.

(4) Thomas, R. D. Salmonella–Menance or Myth? Feedstuffs, January 3, 1970.

(5) U.S. Department of Health, Education, and Welfare, Public Health Service. National Communicable Disease Center Food-Borne Outbreaks Ann. Summary, 1969, pp. 3, 4, 6.

(6) U.S. Department of Health, Education, and Welfare, Public Health Service. National Communicable Disease Center Salmonella Surveillance Monthly Rpts. 1965 to 1970.

(7) Wilson, S. T., Pfow, C. J., and Walker, J. W. Status of the Cooperative State-Federal Salmonella Program. Presented at the 73d Annual Meeting of the U.S. Animal Health Association, October 1969, p. 4.

THE PUBLIC HEALTH SIGNIFICANCE OF SALMONELLOSIS

Richard L. Parker[1]

Summary

Salmonellosis is a major public health problem in the United States, possibly infecting 2,000,000 persons each year. About 30 percent of the outbreaks reported during the past 7 years were poultry or egg associated. Attack rates are highest in the very young, but slight increases above the overall attack rates are noted in the child-rearing and geriatric ages.

Salmonellosis is a public health problem of considerable magnitude in the United States today. The absolute size of the problem can only be estimated, but extrapolation from the reported incidence, based on a working knowledge of the vagaries of reporting of these infections, suggests that two million cases occur annually in the United States, making salmonellosis one of the most prevalent bacterial infections of humans.

In discussing the prevalence of any disease many factors must be considered in determining the overall importance of the affliction. The range of clinical severity will influence the rate at which cases are seen by physicians. The diagnostic accumen of the physician will not only influence recognized morbidity rates but will also determine whether or not other cases are sought in the same group, usually in this instance the family, but the group may often be a diverse mixture of individuals drawn together for a variable period of time for some purpose. The legal status of the disease, i.e., whether or not it is locally legally reportable, will influence the flow of information on cases to a central compiling place. And perhaps one of the most significant factors in knowing the true prevalence of a disease is the current interest in the problem. I would like now to relate these points to salmonellosis.

Humans infected with salmonella organisms, and for this discussion I am not including *Salmonella typhi* infections, may have no symptoms, may have a brief episode of diarrhea, or may need to be hospitalized. A few patients may actually die, although the reported case fatality ratio in the United States is less than 0.3 percent. It is reasonable to ask, "How many of the transient diarrheas are apt to be reported?"

Diagnosis is essential to reporting, but salmonellosis can be confused with a wide number of other gastrointestinal upsets. Salmonella gastroenteritis is considered to be a self-limiting disease, and, although often used, antibiotics are of little value. However, in most instances almost any treatment will seem to bring relief, so why report a resolved nonspecific problem? Far too few busy practitioners have been indoctrinated in the day-to-day workings of epidemiologic procedures; rarely does the clinician look beyond the case or ask "are there others?"

Salmonellosis is reported weekly by the Center for Disease Control, based on the number of isolations reported by State health departments and certain cooperating laboratories. To be reported, an appropriate sample must be collected and submitted to a laboratory which will forward either information or cultures for typing to a laboratory cooperating in the program of national surveillance.

Interest in a disease will often determine the effort put into reporting. For example, a few years ago measles was considered to be a serious problem only when it led to a post-infection encephalitis. It was then assumed that almost everyone contracted the infection sometime during the first 15 years of life; hence the expected annual incidence would have been the total population under 15 divided by 15. In actual practice a small percent of this number of cases was reported. What is the general interest in reporting salmonellosis?

In 1969, the reported incidence rate of human salmonellosis was 10.6 per 100,000, or about 1 per 10,000. The phenomenon of under reporting presented suggests that we may be lucky if 1 or 2 percent of the cases were reported, and, if this be true, the actual incidence of clinical salmonellosis in the United States may be in the magnitude of 1 case per 100 persons per year. This must be considered a public health problem of some importance.

[1] Assistant chief, Office of Veterinary, Public Health Services, National Center for Disease Control, Atlanta, Ga.

Outbreaks of salmonellosis are investigated when they are identified and when resources for investigation are available. Some investigations in which CDC personnel have participated are of special interest, because they illustrate certain factors of surveillance or of an epidemiologic nature. A few months ago an alert local health unit noted the isolation of a Group D salmonella from a woman who had recently returned from a national convention. The woman reported that several others in attendance at the convention from her State had also been ill. Subsequent investigation disclosed that the meal epidemiologically linked to the outbreak was served to about 1,900 convention delegates. A survey was conducted and 27.7 percent of the 440 persons completing the questionnaire were considered to have been clinically ill. *Salmonella panama* was recovered from more than 20 individuals that were or had been ill. Although improperly prepared turkeys were epidemiologically determined to be the source of the infections, this could not be confirmed at the time of the investigation.

In another turkey-associated outbreak, 98 persons became ill after a church supper. *Salmonella saint-paul* was recovered from patients and from the turkey. In a most unusual home outbreak, all 17 persons who ate a grossly undercooked turkey became ill, and two died. *S. enteriditis* was isolated, not only from the patients and the food, but also from a dog that became infected and died.

In the past 7 years about 30 percent of the outbreaks in the United States were traced to infected poultry or eggs; however, since a source cannot always be identified, this is a conservative figure. After several months of freedom from egg-associated outbreaks, a recent investigation incriminated "dirty" eggs as the source of salmonellosis transmitted in eggnog. Turkeys are now emerging as the most important vehicle of poultry-borne salmonellosis.

Epidemiologic studies have defined certain aspects of human salmonellosis. Case rates are high for infants, 97 per 100,000 population under 1 year of age in 1969. By 10 years of age, the age-specific attack rate has dropped to about 5 per 100,000 and remains more or less at this level, with a slight rise probably associated with child rearing and another reflecting the lowered resistance of the aged.

The program of this workshop is clearly directed at resolving the problem of salmonellosis as a threat to public and animal health. Considerable effort is being directed toward eliminating the source of the infection, i.e., toward presenting salmonella-free products to the consumer. However, another point seems worthy of consideration. A large number of salmonellosis outbreak investigations indicate that a lack of technical knowledge or supervision during the preparation of those foods most apt to be contaminated results in the opportunity for bacterial amplification and human infection. The large outbreaks are most noticeable, but what about the frequency with which small family-type outbreaks occur? Is it possible that a campaign to educate all food handlers, including housewives, might reduce human infection? Is it possible that industry could meet this challenge?

FIELD AND CLINICAL ASPECTS OF PARATYPHOID INFECTIONS

Robert W. Keirs[1]

Paratyphoid infection is an important disease of young poultry. Because of its clinical aspects and the age at which it primarily occurs, it can easily go undetected, masked by or lumped in the category of "starve-outs."

Age at Which Infection Occurs

In normal flocks after placement and during the first 10 days of brooding a pattern of early mortality is experienced. It is rather high the first 24 hours, lower the 2d day, increases and reaches a peak at 4 to 5 days, then decreases to a low at 8 days and of no consequences at 10 days of age. This is called the "starve-out" period. Normally the loss is in the range of 1 to 2 percent of all birds placed. It's generally accepted that this loss is unavoidable and includes culls and those birds unable to acclimate or find feed and water.

It is during this same period that the majority of paratyphoid infections are expressed as a disease. The "starve-out" period is extended perhaps by 4 or 5 days, mortality peaks are higher and the percentage of mortality reaches 4 to 8 percent and at times as high as 16 percent. The disease often runs its course by the 14th day and rarily is expressed as a disease pass the 21st day of age.

Incidence by Age

In 1968 at the Western Poultry Disease Conference I gave a report on 1,141 cases of paratyhpoid infections. It covered the years 1960 to 1966, inclusive, and represented four geographical areas of intense poultry production. The age at which these cases were submitted to the diagnostic facilities was documented 707 times; 60 percent of these were submitted before the 21st day; 44 percent, 10 days and younger; 24.5 percent, 5 days and younger; 6 percent on first day. In one geographical area, the age of submission is documented 216 times for the last 6 months of 1968, all of 1969, and the first 6 months of 1970. One-day-old submissions equal 26 percent of the total, while ages from 1 day to the 21st day equal 50 percent.

Incidence by Percentage of Cases Submitted

Incidence of paratyphoid infection, as measured at a diagnostic facility, compared to total cases submitted are as follows: For the four geographic areas during the years 1960 to 1965, of approximately 30,000 cases paratyphoid was diagnosed 812 times, or 2.7 percent. In area #3 for years 1963 and 1964 the incidence was 5.2 percent, or 208 cases, of approximately 4,000 submitted. In this same area for years 1968-69-70 of 5,800 cases it was 3.7 percent. Yet in my own activities for the last 6 months of 1969 and the first 6 months of 1970 of approximately 630 cases only two isolations of paratyhpoid were made. Both cases were in birds less than 10 days of age. The later activities are primarily confined to a monitoring program within a constant population of approximately 1,000,000 broiler hens and pullets.

Symptoms and Lesions

This disease in young poultry appears similiar to many other problems seen during the first week of life. Affected birds are rough feathered, listless, dehydrated, and irritable.

No particular lesion or lesions are consistent. Birds are often semiemaciated with discoloration of musculature and regression of fat. They often have large unabsorbed yolk sacs containing discolored, dirty yellow, sometimes cheesy yolk material. Cecal cores may or may not be present. Often small stellate areas of focal necrosis are seen in the liver. Sometimes perihepatitis, pericarditis, and air sacculitis are seen. The exudates are "dirty"-tinged in contrast to those of *E. coli.* Less frequent findings are hypopyon and synovitis.

Recovery of Organisms

Paratyphoid organisms are readily recovered from all tissue showing lesions. They have also been recovered from bone marrow, lung, skin, and sinus. In 120 cases where tissues other than intestines were the source of isolation, 889 attempts yielded 886 isolations.

With the exception of yolk sacs and intestines, the paratyphoid organisms are often recovered in what

[1] Avian Services, Bentonville, Ark.

seems to be pure cultures. The yolk sac may hold the clue to one source of infection for from it usually five or more organisms are recovered. They are Escherichia coli, Pseudomonas sp, Proteus, and Strep. fecalis besides Salmonalla sp.

Hatching Eggs as a Possible Source of Infection

In poultry production the care and handling of hatching eggs has been slighted for years. Recently emphasis has been placed on upgrading this phase of production. Floor eggs are a common management problem. Such eggs become readily soiled with dirt and fecal matter. Many eggs are still collected in wire baskets; this can result in high numbers of cracked eggs, which lead to internal contamination. Daily egg pickups are often too few, and on-the-farm fumigation is an uncommon practice. Egg rooms are too often filthy, or a repository for junk. Nest boxes are often too few, in poor repair, and lacking in adequate clean nesting materials. These practices lead to soiled and damaged hatching eggs. Soiled eggs are at times wiped with damp rags. It is possible that this phase of production could be contributing to the incidence of paratyphoid infection.

SUMMARY OF RECENT SALMONELLA RESEARCH AT UNIVERSITY OF CALIFORNIA, DAVIS

W. W. Sadler[1]

Groups of chickens aged 2 days and 1, 2, 4, and 8 weeks were inoculated orally with 10^2, 10^4, 10^6, 10^8, or 10^{10} viable cells of a strain of *Salmonella typhimurium*. They were maintained in wire-floored cages in isolated quarters, and cloacal swabs were taken from each bird and cultured for salmonellae at selected intervals from the third to the 94th day. Level of intestinal infection, as evidenced by fecal shedding of viable salmonellae, was directly correlated with bird age and inoculum dose. Stressing nonshedding birds did not influence the shedding pattern significantly. Only 8 percent of all inoculated birds were still shedding on the 38th day, only 1 percent on the 73d, and 0.5 percent on the 94th. Necropsies with bacteriological culturing between the 77th and 115th days revealed intestinal infection in one bird that had shed continuously and in six that had stopped shedding 22 to 79 days previously. Mortality was 25 to 50 percent in the 2-day-old birds, but no deaths attributable to salmonella infection occurred in the older groups. Repetition of portions of the experiment with a recently isolated field strain of *S. typhimurium* gave comparable results.

Sampling of 39 sites over the entire intestinal tract revealed infection in 14 of 50 experimental birds at necropsy. The number of sites from which isolations were made in any one bird ranged from 1 to 29. Each of the 39 sites yielded salmonellae in at least one bird. Sampling of cecal contents and cecal tonsils was superior to sampling either cecal contents or, particularly, cecal tonsils alone.

Birds inoculated orally with *Salmonella typhimurium* and deprived of water for various periods had comparable infection rates but marked differences in longevity of shed. Longevity of shed was influenced most when water deprivation was during the 3 days preceding inoculation. Infection rates and shed duration were greatest from *S. typhimurium* culture administered orally plus *Escherichia coli* given intramuscularly, less from *S. typhimurium* culture orally plus magnesium sulfate, and least from *S. typhimurium* given alone or from washed cells of *S. typhimurium* given orally.

When infected birds were held on wire until only 1 of 42 continued to shed, and then transferred to a litter floor, 60 percent shed one or more times in the following 28 days. Sixty-three days after transfer, however, all birds and all litter samples were negative; and no salmonellae were isolated at necropsy. Extensive sampling of litter failed to reveal salmonellae, and baby chicks brooded on this litter and sampled for 5 weeks failed to become infected.

Preliminary studies with *Salmonella infantis* and *S. typhimurium* showed that those salmonellae do not persist as long in builtup poultry litter as in fresh litter. The shed rate of birds on builtup litter suggests an inhibitory effect for salmonellae in the litter. This effect may reduce salmonellae in either the litter or the intestinal tract. Cycling of salmonellae between litter and intestinal tract appears of significance in maintaining intestinal infection. This cycling is more evident in unchanged new litter than in builtup litter or in fresh litter changed periodically. Fresh litter replaced periodically did not appear to differ from builtup litter in infection rate. Contamination levels of the two litters were similar.

A safe and effective method of local anesthesia of chickens with Xylocaine was demonstrated. All other anesthetics tested were unsatisfactory because of a slow induction period, struggling, and resultant mortality. Various surgical procedures and techniques including laparotomy, cecal ligation, cecostomy, and cecectomy were tested. With cecal ligations and cecectomies the cecal pouches were not completely eliminated. The feasibility of successfully producing intestinal fistulas was demonstrated.

Surgically altered and normal chickens were inoculated orally with *Salmonella typhimurium* and, in some instances, ferric oxide, and intestinal infection was monitored by sampling feces and cecal contents. Salmonellae initially appeared in feces in 1.5 to 22 hours, and ferric oxide in 3 to 5 hours. The organisms could not be isolated from the ceca until 24 hours. Birds whose ceca had been ligated or surgically removed had a higher shed rate than the controls. No evidence indicated that the cecum plays an essential role in establishing or maintaining intestinal infection with *S. typhimurium*.

[1]School of Veterinary Medicine, University of California, Davis.

Surgically bursectomized and control birds were inoculated intraperitoneally with *S. typhimurium* and necropsied at 3 to 48 hours. Counts of salmonellae in artifically induced abdominal fluid at necropsy revealed significantly larger numbers in bursectomized than nonbursectomized at 6 hours; this indicates an active role for the bursa of fabricius in the immune response to parenterally inoculated salmonellae.

S. typhimurium was inoculated via the rectum into the ceca of bursectomized and control birds. Counts of salmonellae in cecal contents at 24 and 48 hours revealed no difference between the groups. Fluorescent antibody examination of frozen sections of cecal walls, however, revealed a statistically significant higher rate of tissue invasion in bursectomized as compared with nonbursectomized birds.

Groups of bursectomized, thymectomized, and control birds were inoculated orally with either 10^4 or 10^8 organisms of *S. typhimurium* and inoculated intraperitoneally with 10^6 organisms 48 days later. Rectal swabs taken at intervals following the two inoculations provided further evidence that the bursa of fabricius plays a significant role in the immune response of chickens to *S. typhimurium*. No difference was noted between the thymectomized and control birds.

Research Proposed at University of California, Davis

Information will be developed in the following areas:

(1) The effect of microbial competition by organisms, particularly other enteric microorganisms, on the growth of salmonellae in litter and in the intestine of the bird; (2) the origin of any naturally occurring salmonellae-inhibiting substances in the litter and the influence of these natural inhibitors and intentional additives on the growth of salmonellae in litter; (3) the influence of type of litter on the growth of salmonellae and other microorganisms in litter; (4) the survival of salmonellae in naturally contaminated vs. artificially inoculated litter, both new and old; (5) methods of controlling the litter on which birds are housed to insure a salmonellae-free flock at the time of slaughter; (6) field studies on the effect of several different litters and of various manipulations and treatments of litter on infected poultry from the time of hatch until slaughter; (7) field studies on the influence of various other husbandry practices and control procedures on establishment and maintenance of salmonellae-free flocks under commercial conditions.

CYCLE OF SPREAD OF SALMONELLAE IN POULTRY

George K. Morris[1]

There have been many investigations and numerous reports on salmonellae in commercial poultry. Some of these reports have dealt with epidemiological aspects of this problem. Epidemiological studies on the transmission of salmonellae in integrated broiler operations have been conducted for several years by the Center for Disease Control (formerly the National Communicable Disease Center) in collaboration with commercial poultry concerns. In these studies, both the open cycle and the closed cycle of spread have been demonstrated.

A closed-cycle type of spread was demonstrated by Kaufmann and Feeley[2] in a Salmonella culture survey of a vertically integrated broiler operation. A self-perpetuating cycle of infection was indicated by isolation of the same serotypes from the live birds, their feed, and the poultry meal incorporated into the feed. The poultry meal, which was rendered offal from the company's own processing plant, served as the major source of feed contamination. Salmonellae were isolated from 95 percent of 48 poultry meal samples examined. Another major source was the fish meal added to the feed (44 percent of 34 samples). The hatcheries appeared to be unimportant in the perpetuation of infection in this operation.

An open-cycle type of spread was noted in a study conducted by CDC in collaboration with B. L. McMurray of Central Soya Company, Inc.[3] Salmonellosis, apparently causing chick mortality, was identified in several broiler flocks. Serotypes of the organism similar to serotypes identified in the broilers were traced to the feeds and to the breeder hens from which the broilers originated; this indicates two possible sources of infection. The same serotypes were identified in the broilers when slaughtered 5 to 7 weeks later.

Feed ingredients most often contaminated were those of animal origin. Nonpelleted feeds were more frequently contaminated than pelleted feeds. Poultry byproducts in this company were not processed in the company's own facilities; therefore, the cycle of spread was not completed as in the study by Kaufmann and Feeley.

One flock of breeder hens was found to excrete Salmonella throughout their entire growing and laying period of about 460 days. The infection rate was highest in this flock when they were yound chicks, followed by another peak of high infection rate when the hens were moved to the laying farm at maturity. A study of broiler flocks originating from this breeder flock indicated that, in this case, the infection was not transmitted to the progeny.

In both of the above studies the hatchery did not appear to play an important role, but another study at CDC (unpublished data) revealed that *Salmonella infantis* was perpetuated within the hatchery, with the result that about 50 percent of the chicks hatched were contaminated. In this study, the serotype isolated from the hatchery, the young chicks, and the broilers when processed was not present in eggs or in the breeder hens from which the eggs originated.

Examination of carcasses at various stages of processing indicated points in processing at which decontamination and recontamination of carcasses occurred. Spray washes were very effective in the decontamination of carcasses. Recontamination of carcasses occurred during evisceration and during chilling.[4]

These and other studies indicated many ways by which poultry can become contaminated with salmonellae. Since the level of contamination and the method of spread cannot always be predicted, except by bacteriological studies of each operation, it is very important that each integrated poultry operation have access to bacteriological laboratory services so that the potential salmonella hazards can be properly evaluated.

---

[1] National Center for Disease Control, Atlanta, Ga.

[2] Kaufmann, A.F., and Feeley, J.C. Culture Survey of Salmonella at a Broiler Raising Plant, U.S. Pub. Health Serv., Pub. Health Rpt. 83: 417-422. 1968.

[3] Morris, G.K., McMurray, B.L., Galton, M.M., Wells, J.G. A Study of the Dissemination of Salmonellosis on a Commercial Broiler Chicken Operation. Amer. Jour. Vet. Res. 30:1413-1421. 1969.

[4] Morris, G.K., and Wells, J.G. Salmonella Contamination in a Poultry Processing Plant. App. Microbiol. 19: 795-799. 1970.

DYNAMICS OF SALMONELLA INFECTION IN FRYER ROASTER TURKEYS

M. C. Kumar, M. D. York, J. R. McDowell, and B. S. Pomeroy[1]

Summary

A comprehensive study of salmonella infections in nine fryer roaster turkey flocks was carried out in Minnesota involving eight different farms and three hatcheries. There were approximately 119,000 birds in these flocks.

Results of the study on three of the flocks were reported in detail. The sampling procedures and types of samples obtained were considered to be sufficient for monitoring flocks for salmonellosis. The sampling of feed was not considered adequate in this study.

Three sources of salmonella infection were disclosed in this study viz: (1) Breeder flocks and hatcheries, (2) contaminated environment of turkey houses, and (3) contaminated feed. Contaminated environment of the turkey houses invariably infected the birds once the birds came into contact with the environment. Two of the three flocks were infected by salmonellae present in the environment. *Salmonella kentucky* was isolated both from the feed and the birds at the same time, which shows a direct relationship between feed and infection in the birds. Feed was a possible source of infection in two of three flocks reported in this study. Source breeder flocks and hatcheries were responsible for salmonella infection in the poults in two of three flocks. Of the nine flocks studied, one was negative for salmonella, and the sources of *Salmonella* infection in the rest of the flocks were: environment (three flocks), environment and feed (one flock), environment and breeder flocks and hatchery (one flock), breeder flocks and hatchery and feed (one flock), breeder flocks and hatchery (one flock), and unknown source (one flock).

Review of Research in Progress

Research on salmonellosis in turkeys is directed toward the following aspects:

(1) Cycle of salmonella infection from breeders to poults and the role of hatchery in dissemination of salmonellae.

A turkey hatchery was selected and is being monitored for salmonellae. All its breeder flocks are under surveillance, and samples are collected periodically from the flocks and their environment. Replacement breeders are also monitored for salmonellae. An effort is being made to break the cycle of infection by employing various methods. They include litter management with "Litter Life," better egg sanitation, fumigation of eggs after each collection, frequent collection of eggs, elimination of dirty eggs, good sanitation procedures at the hatchery level, and antibiotic injection of the freshly hatched poults.

Despite all these efforts the cycle of salmonella infection has not been broken. Dipping of eggs in antibiotics is being tried to find out if it is possible to eliminate salmonellae from eggs by this procedure. This has shown promise in the laboratory. A field trial will be conducted with a larger number of eggs.

(2) Types of building materials required for effective elimination of salmonellae from the environment.

A building was constructed with the cooperation of Minnesota Breeder Hen Committee, 3M Company, and Dow Chemical Company. This building is divided into four rooms; each has its own ventilation and heating systems. Dow Chemical and 3M Company constructed the partitions and coated the walls, floors, and ceilings with a compound that was supposed to be impervious to organisms. One trial has been carried out, and the infection stayed confined to the birds in the infected groups. The control groups remained negative. In the future different types of surface material will be tried out in this building to determine how readily the rooms may be decontaminated.

(3) Sources of salmonella infection in fryer roaster flocks.

The purpose of this research project was to determine the level and sources of salmonella infection in fryer-roaster turkeys from time of hatching to the time they arrive at the processing plant. This project is in its second year. Nine flocks have been chosen each year and were followed from the time of hatching to processing.

In the next fiscal year an attempt will be made to find out effective methods of eliminating salmonellae from the environment of the turkey houses.

[1] Department of Veterinary Microbiology and Public Health, College of Veterinary Medicine, University of Minnesota, St. Paul, Minn.

(4) Salmonella contamination in rendering plants.

The sanitation program in various types of rendering plants have been under study in Minnesota for the past 4 years. The purpose of the project is to:

(a) Determine where, how, and why recontamination of the finished animal and poultry byproducts occurs.
(b) Find ways in which recontamination can be minimized.
(c) Develop uniform sampling techniques.
(d) Improve existing laboratory procedures for isolation and identification of salmonellae.

The primary problem in many rendering plants is the inability to establish and apply an adequate sanitation program. Some plants have the capability of producing a negative finished product, others have significantly reduced the contamination rate, but others have made little or no progress toward producing a Salmonella-free product.

Research will be conducted in the following areas.

(1) Efforts will be continued to break the cycle of salmonella infection at the hatchery level.
(2) Evaluate methods of cleaning and disinfecting turkey houses to eliminate salmonellae from the environment.
(3) Conduct experiments to find out if age of infection has any influence on the duration of infection and serological response.
(4) Salmonella contamination of processed turkeys and products will be assessed.
(5) Eggs from primary breeders free of salmonella and arizona infections will be monitored. The premises on which these turkeys will be placed will be extensively sampled for salmonella.
(6) Development of diagnostic procedures to identify carrier flocks.

Recommendations

There is every indication that the incidence of *S. typhimurium* infection in turkeys has been reduced over the past 20 years. This has resulted from concentrated effort by the industry and the Typhimurium Control Program (NTIP) to reduce this serotype in turkey breeder flocks. However, other serotypes, such as *S. saint-paul* and *S. heidelberg*, have not received this same attention and have thus increased in incidence. Hence, total increase of salmonella infections in turkey breeder flocks has occurred. We have reduced one important serotype but have minimized our efforts with the other serotypes.

The question is should our efforts be directed to reduce or eliminate the other salmonella serotypes such as *S. heidelberg* and *S. saint-paul*.

The following five point effort may be made:

(1) Exploit the egg sanitation program

(a) Under the existing control program (NTIP) every effort should be made to get maximum benefit from the egg sanitation and fumigation programs on the farm. Each breeder farm shall be inspected by an agent of official State agency to see that fumigation equipment, procedures, egg collection, and handling programs are being carried out at recommended level.

(b) At the hatchery level the sanitation program for hatching eggs be reviewed with each hatchery and be enforced by periodic inspection program by an agent of OSA.

(2) Deminish the salmonella contamination in the environment

(a) Most of the turkey farms and buildings are contaminated with salmonellae. Work out recommended procedures to decontaminate existing poultry buildings.

(b) Encourage replacement buildings to be so constructed that they can be cleaned and disinfected.

(c) Encourage complete confinement rearing for the entire life cycle of breeder flocks.

(3) Concentrate on certain salmonella serotypes

To reduce the other salmonella serotypes is going to require an attack at the total environment of the breeder flocks.

(a) Identify by serological and cultural techniques the salmonella-infected flocks.

(b) Eliminate typhimurium-infected flocks.

(c) Concentrate efforts to reduce the incidence of other serotypes by the control of the environment and egg sanitation programs and serological and cultural procedures.

(4) Salmonella-free feeds

(a) Encourage control programs in the feed industry to eliminate salmonella contamination of animal feeds.

(b) Feed for breeder flocks be required to be salmonella-free for the life cycle of the flock. A starting point would be to require all feed for use in breeder flocks be heat treated (crumblized, pellet feeds, etc.).

(5) Water supply to breeder flocks to be from a potable source.

LITTER CULTURING AS A MEANS OF DETERMINING THE SALMONELLA STATUS OF CHICKEN FLOCKS

G. H. Snoeyenbos[1]

Litter sample culturing has been developed as a method to identify the salmonella status of chickens reared and maintained on litter. Under many circumstances demonstration of environmental contamination with salmonellae can be used more dependably and economically than other more direct methods of detecting flock infection.

The capability of isolating salmonellae from letter, or other environmental samples, is influenced by multiple factors bearing upon the total number and distribution of viable salmonellae. As a generalization, infection in a group of chicks (reared on new litter) progresses very rapidly throughout the flock; excretion and litter contamination levels are high at this time. As such flocks mature the percentage of infected birds sharply declines as does the number of salmonellae excreted. Long used litter has a decided salmonellacidal effect by a little understood mechanism. This salmonellacidal activity can be sufficient to moderate the rate of salmonella transmission within a flock, to interfere with detection by litter sampling, and to destroy contaminating salmonellae within a few days.

Elevated incubation temperatures (41.5° - 43° C) for either tetrathionate or selenite enrichment broths are gaining wide acceptance for some materials and should be used for isolating salmonellae from the large and varied microflora present in letter. Optimum time for plating appears to be 24 to 36 hours after the medium has reached incubation temperature.

The litter sample periods and the number of samples to be examined must be determined rather arbitrarily dependent upon the management system used and the accuracy desired. Sample collection at 1, 3, and 5 weeks after starting chicks on new litter and at the same intervals following moving to cleaned pens yields a reasonably dependable evaluation of the flock status if they are maintained under adequate security management. More prolonged sampling is sometimes necessary to detect infections resulting from residual pen contamination from previous populations. Sample numbers may be based on the bird population per pen and should be collected from randomly selected, dry, heavily used areas of the pen. Samples of approximately 10 grams, to be cultured in 100 ml. of enrichment broth, are taken from the fine dry particles in the upper 1 to 2 inches of litter. Fresh droppings should be avoided. Considerable reliance can be placed on the results from five samples from pens of up to 500, 10 samples from pens of 500 to 2,500, and 15 samples from larger pen numbers.

Multiple isolations of a single serotype may be interpreted as indicating flock infection. Single isolations may at times represent environmental contamination without bird infection.

A litter sampling program can be used as a practical method of determining the salmonella status of chickens started on new litter. The applicability of this approach for other species has not been documented.

[1] University of Massachusetts, Amherst.

EVALUATION OF SALMONELLA CULTURE PROCEDURES

George K. Morris[1]

It is generally agreed that enrichment of fecal specimens or food samples in some type of broth, either nonselective or selective, is superior to direct plating for isolating salmonellae. This is particularly true with samples from carriers and food samples in which there are only a few salmonellae. It is difficult to recover salmonellae without selective enrichment if the normal coliform outnumber the salmonellae by as little as 10:1.

Three selective media commonly used to recover salmonellae–tetrathionate broth, selenite broth, and gram negative (GN) broth–are frequently used in combination with other selective agents. Ordinary nutrient broth sometimes acts as an enrichment medium for isolating pathogens from feces, and lactose broth is commonly used as a preenrichment prior to inoculation into selective enrichment. Jameson[2] found that a secondary selective enrichment inoculated with a relatively large inoculum from selective primary enrichment increased the yield of salmonellae. In the laboratory at CDC, we have found that the secondary enrichment method utilizing similar medium as that used in the primary enrichment is a useful technique in salmonella studies, especially when the samples are inoculated directly into the enrichment broth in the field and are not delivered to the laboratory for 1 or 2 days.

In table 1 are the results observed when the secondary enrichment technique was evaluated with tetrathionate, selenite, and GN broths. The primary enrichment broth was plated after 48 hours' incubation at 37° C. The secondary enrichment was subcultured from the primary enrichment when the primary enrichment broth was 1 week old. The secondary enrichment was incubated at 37° for 24 hours. In this work, in the tetrathionate broth, two organisms were isolated from the secondary enrichment that were negative by the primary enrichment method; whereas, in the selenite broth, three salmonellae isolated from the primary broth were subsequently found to be negative by the secondary enrichment method. Although these figures are too small to be significant, they do reflect what we have noted in our experiences with these media, i.e., salmonellae grow out faster in selenite broth, but tetrathionate broth is the more stable medium when samples are incubated for several days.

Table 1.–Evaluation of the secondary enrichment technique for isolating salmonellae from human feces with 3 enrichment broths

Enrichment combination	Number positive with–		
	Tetra-thionate	Selenite	Gram negative
Both primary and secondary . .	22	21	17
Primary only.	0	3	1
Secondary only	2	0	1
Total	24	24	19
Total samples examined	49	49	49

An evaluation of brilliant green sulfadiazine (BGS) and xylose lysine desoxycholate (XLD) plating media (table 2) indicated three things: (1) These plating media are equally effective for isolating salmonellae from tetrathionate broth, (2) salmonellae appear to be overgrown by competing bacteria in the relatively nonselective GN broth, and (3) the highly selective BGS agar is more effective than the less selective XLD agar when used in combination with GN broth. Also, salmonellae tend to become sensitive to BGS agar after prolonged exposure to selenite broth, as can be noted with the results of the secondary enrichments.

The advantage of a secondary enrichment for isolating salmonellae in tetrathionate broth cultures of human and chicken feces (table 3) is shown by the fact that seven isolations were made from the secondary broths that were negative by the primary enrichment method.

Further advantages of this technique were noted when tetrathionate broths were inoculated with samples in the field and were not brought to the laboratory for 1 to 2 days (table 4). Fifty-six samples were positive by

[1] National Center for Disease Control, Atlanta, Ga.

[2] Jameson, J.E., A Study of Tetrathionate Enrichment Techniques, With Particular Reference to Two New Tetrathionate Modifications Used in Isolating Salmonellae From Sewer Swabs. Jour. Hygiene 59:1-13. 1961.

Table 2.–Evaluation of BGS agar and XLD agar for isolating salmonellae from three enrichment broths

Medium	Number positive from–					
	Primary enrichment broths			Secondary enrichment broths		
	Tetrathionate	Selenite	Gram negative	Tetrathionate	Selenite	Gram negative
BGS and XLD	22	18	6	23	13	11
BGS alone	0	4	[1]12	1	1	[1]7
XLD alone	0	2	0	0	[2]7	0
Total BGS	22	22	18	24	14	18
Total XLD	22	20	6	23	20	11
Total both media	22	24	18	24	21	18

[1] Significantly greater than 0.
[2] Significantly greater than 1 (P less than 0.10).

Table 3.–Evaluation of the secondary enrichment technique for isolating salmonellae

Enrichment combination	Number positive in–		
	Human feces	Chicken feces	Total
Both primary and secondary	22	11	33
Primary only	0	0	0
Secondary only	2	5	7
Total	24	16	40
Total samples examined	49	300	349

the secondary enrichment method and negative by primary enrichment; whereas, only seven were positive by primary enrichment and subsequently found negative by secondary enrichment.

These studies indicate the usefulness of secondary tetrathionate enrichment cultures, especially when the samples are inoculated directly into the enrichment broth in the field and this inoculation precedes arrival at the laboratory by 1 to 2 days.

Table 4.–Evaluation of the secondary enrichment technique for isolating salmonellae when samples held 1 to 2 days before arrival at the laboratory

Enrichment combination	Number positive in–		
	Human feces	Fish meal	Total
Both primary and secondary	78	148	226
Primary only	0	7	7
Secondary only	9	47	56
Total	87	202	289
Total samples examined	404	684	1,088

WHAT IS NEW IN SALMONELLA BACTERIOLOGY?

E. M. Ellis[1]

I would like to very briefly review some of the newer ideas for you and then just add a word about FA conjugate and close with a discussion of a simple statistical method.

The order in which I present these ideas in no way reflects their importance.

Taylor *(9)* has developed several new plating mediums. Two of these that became of interest to us were XLD and XLBG agars. These were designed primarily for shigella. XLD agar contains xylose, lysine, desoxycholate, and other chemicals. Salmonella colonies in 24 hours should appear red and lactose fermenting organisms yellow. XLBG, on the other hand, has brilliant green added.

We have evaluated both of these agars *(4)*. XLD agar has been in use in our Salmonella Reference Center. According to the literature, salmonella colonies should appear red in 18 to 24 hours. Our experience has been that these are yellow in 18 to 24 hours, which indicates a lactose fermenter, and red in 48 hours. The medium has offered no advantages to us over BGS agar. We have not tested it extensively. The XLBG agar was studied in a limited experiment. The results are represented in table 1. You can see that this agar offered no particular advantage over BGS. The difference noted is not significant. Our staff agrees that where one is working with enteric organisms originating from man and primates, these mediums would be much more useful.

Table 1.–Comparison of XLBG[1] and BGS[2] for the isolation of salmonella from 224 swine cases

Method	Positive		Negative	
	Number	Percent	Number	Percent
XLBG	53	23.7	171	76.3
BGS	55	24.6	169	75.4

[1] Xylose-lysine-brilliant green.
[2] Brilliant green-sulfadiazine.

Another medium used recently by Smyser and coworkers *(8)* (table 2) is selenite brilliant green sulfapyridine broth (SBG). This enrichment was compared with tetrathionate brilliant green (TBG) broth preceded by lactose preenrichment. TBG broth was incubated at 37° C., SBG sulfa at 37° and at 43°. Tetrathionate was not compared at 43°. In table 2 can be seen the results of culturing animal byproducts, grain, plant protein, and complete feed. TBG at 37° gave 25 positives, SBG sulfa at the same temperature gave 30 positives, and SBG sulfa at 43° gave 36 positives for animal byproducts. The 43° temperature incubation needs further study. We have experienced difficulty with

[1] Chief, Diagnostic Bacteriology, National Animal Disease Laboratory, Ames, Iowa.

Table 2.–Isolation of salmonella from 227 samples of animal feedstuff cultured in SBG[1] sulfa and lactose preenrichment-TBG[2] group I[3]

Sample	Number tested	Number positive	Number positive in–		
			TBG 37° C.	SBG-sulfa 37° C.	SBG-sulfa 43° C.
Animal byproducts	86	46	25	30	36
Grain	34	0	0	0	0
Plant protein	34	1	1	0	0
Animal feed	73	2	1	0	1
Total	227	49	27	30	37

[1] Selenite brilliant green sulfapyridine
[2] Tetrathionate brilliant green
[3] From *(8)*.

it due to inhibition of motility of salmonellae when grown at this high temperature. The other results are probably not significant.

A new agar was described recently by Docksteader at the ASM meeting in Boston *(7)*. He named it PDS agar, for Padron-Docksteader Salmonella agar. The agar in his hands has proved to be valuable in shortening the time for recognition of salmonellae. It is inoculated at the same time as the triple sugar iron (TSI) slant. It is recommended that, with a straight needle and culture, the user stab the center of the agar butt and move the needle laterally to the wall of the tube, creating a slash inoculation site. Incubate at 35° to 37° C. A positive reaction is the appearance of a black pigment diffusing from the site of inoculation. It is stated that PDS agar positive reactions occur in as little as 3 hours incubation. According to the authors, it will identify most salmonellae, arizonae, and edwardsiella.

A scheme for identifying salmonella antigens in foods was presented recently by MacFarland *(6)*. Briefly, the samples were cultured in a chamber separated by a filter. The salmonellae grew and passed through the filter membrane from the inoculum side to the sterile broth on the opposite side. The antigen obtained was heated at 60° C. and used as the antigen in a double gel diffusion test. A capillary tube was used for the reacting system. Anti-salmonella globulin served as the antibody. A ring of precipitate formed in the noble agar of the arena was considered a positive test. Several techniques were described including the use of a "tea bag" to hold the cultured sample. We are using a "tea bag" technique where the feed sample is cultured inside a porous bag submerged in the broth medium on a trial basis.

I will make a few remards about an FA conjugate prepared by Ellis and Harrington *(2)* that is now being used in some Animal Health programs. The conjugate is chiefly an "H" conjugate but contains a slight contaminating amount of "O" antibody. "H" conjugates prepared in rabbits should probably be referred to as "O" "H" conjugates even though the amount of "O" antibody is very small and when diluted out in the test is present in minute amounts. Goepfert *(3)* has indicated that the somatic staining seen in so-called "H" preparations may, in fact, be due to contaminating "O" antibody.

With our conjugate, we are at least 98 percent confident that the viable salmonellae, if in the sample, will be detected, and at least 95 percent confident that if organisms fluoresce, they will in fact be salmonellae. Our protocol is available upon request for those interested. We employ a flazo orange counterstain which stains debris and non-salmonellae orange in contrast to the yellow-green color of the fluorescein. We also use a product called Fluoroglide with which we prepare our slides.

Harrington and Moulthrop *(5)* compared the FA technique, selenite-F broth culture, and gram negative broth culture. In table 3 are the results of culturing 118 chicken livers for salmonella. These figures do not indicate the superiority of one enrichment broth over the other. The FA technique is more sensitive than cultural methods that involve plating and biochemical identification. Our studies using FA are too numerous to mention in the time allowed for this presentation.

Table 3.–Comparison of the FA technique, selenite-F culture, and GN culture for salmonella on 118 chickens livers[1]

Method	Positive		Negative	
	Number	Percent	Number	Percent
FA technique .	42	35.6	76	64.4
SF[2] culture . . .	38	32.2	80	67.8
GN[3] culture . .	32	27.1	86	72.9

[1] According to Harrington and Moulthrop.
[2] Selenite-F broth.
[3] Gram negative broth.

I will conclude with a little philosophy. For the past 10 years, we have been reading in the literature all sorts of comparisons of one medium with another and one technique with another. I wish to ask the question here as to whether we are aware that we have made a number of assumptions when we say a culture is positive or negative.

Generally, when an examination for salmonella is begun, we put X grams of liver, feed, or animal byproduct into a certain amount of an appropriate broth and proceed to attempt the isolation of salmonellae from the sample. When we do this, we have made, or must be willing to make, certain assumptions:

1. That there is homogeneity of the organism in the sample.
2. That the sample is a randomly selected one.
3. That a given number of organisms per 100 grams represent the lower limit of expected recovery. For example, we are willing to say that if two salmonella per 100 gram are present, we expect to isolate them.

The question should arise as to how much of the material we must culture to expect success. For example:

1. Given a lower limit of two organisms per 100 grams.
2. A homogeneous mixture.
3. A random sample.

How much material must we culture to be 95 percent confident that if there are two organisms per 100 grams of sample, we will be successful. If we use the formula adopted from Beazley *(1)*, we find:

$$N = K/C$$

When: N = total number of grams of sample that must be tested.

C = contamination level against which protection is desired. This is expressed as the number of organisms per 100 grams of material.

K = a factor derived from statistical tables and relates to the reliability level or the percent probability of detection. K is found from tables such as the following:

Level of reliability	*K*
Percent	
50	70
80	161
90	230
95	300
99	460

95 percent reliability desired

N = K/C from table

find K = 300

N = 300/2

N = 150 grams of sample tested, or six 25-gram samples

I would urge all who now entertain the idea of doing comparative testing of mediums and methods to be sure of the assumptions made and then to culture an amount of material, defined by a dependable statistical method.

Literature Cited

(1) Beazley, C. C., and Insulata, N. F. The Roles of Fluorescent Antibody and Statistical Techniques for Salmonellae Protection in the Food Industry. Inst. Food Technol. Ann. Meeting, San Francisco, Calif., May 24-29. 1970.

(2) Ellis, E. M., and Harrington, Rube. A Direct Fluorescent Antibody Test for Salmonella. Arch. Environmental Health Vol. 19. 1969.

(3) Goepfert, J. M., and Hicks, Ruthann. Immunofluorescent Staining of *Salmonella* Species with Flagellar Sera. Appl. Microbiol. Vol. 18. 1969.

(4) Harrington, Rube, and Ellis, E. M. Comparison of Two Plating Media for Detection of Salmonella from Swine Tissues. Appl. Microbiol. Vol. 20. 1970.

(5) _____ and Moulthrop, I. M. [In Press.]

(6) McFarlane, J. O., Bailey, C. A., Ackerson, D., and others. A Unique Two-Phase Rapid Technique for Salmonella Detection in Foods. Amer. Soc. Microbiol., Boston, Mass. 1970.

(7) Padrone, A. P., Wilson, C. R., Docksteader, W. B. Selective Medium for H_2S Production by Salmonella. Bact. Proc. p. 6 1970.

(8) Smyser, C. F., Snoyenbos, G. H., and McKie, Barbara. Isolation of Salmonellae from Rendered By-Products and Poultry Litter Cultured in Enrichment Media Incubated at Elevated Temperatures. Avian Dis. 14 (2). 1970.

(9) Taylor, W. I. Isolation of Shigellae, 1. Xylone Lysine Agars; New Media for Isolations of Euteric Pathogens. Amer. Jour. Clin. Path. No. 4. 1965.

STATUS OF SALMONELLA ACTIVITIES OF THE ANIMAL HEALTH DIVISION, ARS, USDA, AND THE COOPERATING STATE AGENCIES

Saul T. Wilson, Jr.[1]

The major activities directed toward the elimination of salmonella in livestock and poultry by the Animal Health Division, Agricultural Research Service are: (1) The cooperative State-Federal industry program to eliminate the transmission of salmonella through livestock and poultry feeds at the processing level; (2) salmonella diagnostic assistance to State, industry, and university-supported animal disease diagnostic laboratories.

State-Federal Program

The immediate objective of the State-Federal industry salmonella program is to eliminate transmission of salmonella through animal and marine proteins used in livestock and poultry feeds. The program is voluntary and involves the State agency that has regulatory control of plants processing animal and marine proteins. All 50 States and Puerto Rico are participating in the program.

The program calls for plant inspection, product sampling, and in-plant surveys by State and Federal personnel for sources and practices that might lead to salmonella contamination of the plant's finished product. The Sanitation Guidelines for processors of animal byproducts[2] and industrial fishery products[3] are basic standards for plant inspections and for managers to apply in their daily operations.

The program is designed to go through three phases on an individual plant basis; Phase I "Evaluation," Phase II "Clean-up," and Phase III "Approved." The ultimate objective for each participating plant is "Approved Status." This status is attained when the plant has had the required number of negative tests and has implemented a management-operated Salmonella control program that is approved by the cooperating State-Federal officials.

Bacteriological monitoring is an integral part of the management-operated programs. The purpose of these programs is to minimize the opportunity for salmonella contamination of a plant's finished product and to have a routine procedure to detect it when it occurs. Emphasis is upon preventative practices in a plant's daily operation. The activities of State and Federal personnel in approved plants will be limited to verifying the adequacy of a plant's own salmonella control program.

At the close of fiscal year 1969, 78 percent, or 728, of the 930 plants in the United States processing feed supplements of animal and marine origin had been evaluated. Of the evaluated plants 27 percent are in the negative category. (The program definition for a negative plant is one in which salmonella was not detected in 30-consecutive-sample units of finished product collected from the shipping or storage area by a State or Federal inspector. The 30-sample units must be collected on three or more plant visits within a 1- to 12-month period with not more than 10-sample units being collected on any one visit.)

A summary of the results of examinations of finished product samples for salmonella in fiscal year 1969 is given in figure 1. Forty percent of the samples collected in plants classified as blenders were positive. This is more than twice the percentage of positive samples obtained in either of the other categories of plants. The percentage of samples positive for rendering plants that are part of a poultry-slaughtering establishment did not differ significantly from the percentage of samples positive for rendering plants that are part of a livestock-slaughtering establishment.

Figure 2 gives the number of plants evaluated by plant category at the close of fiscal year 1969, and the percentage that met program requirements for negative status. None of the 11 evaluated plants in the blender category had a sufficient number of negative tests for designation as a negative plant, while 55 percent of the 63 evaluated marine plants met the requirements for a negative plant.

---

[1] Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture, Federal Center Building, Hyattsville, Md.

[2] U.S.D.A., Agricultural Research Service. Recommended Sanitation Guidelines for Processors of Poultry and Animal By-Products. U.S. Agr. Res. Serv. ARS 91-47. 1966.

[3] U.S.D.A., Agricultural Research Service. Sanitation Guidelines for Salmonella Control in Processing Industrial Fishery Products. U.S. Agr. Res. Serv. ARS 91-51. 1949.

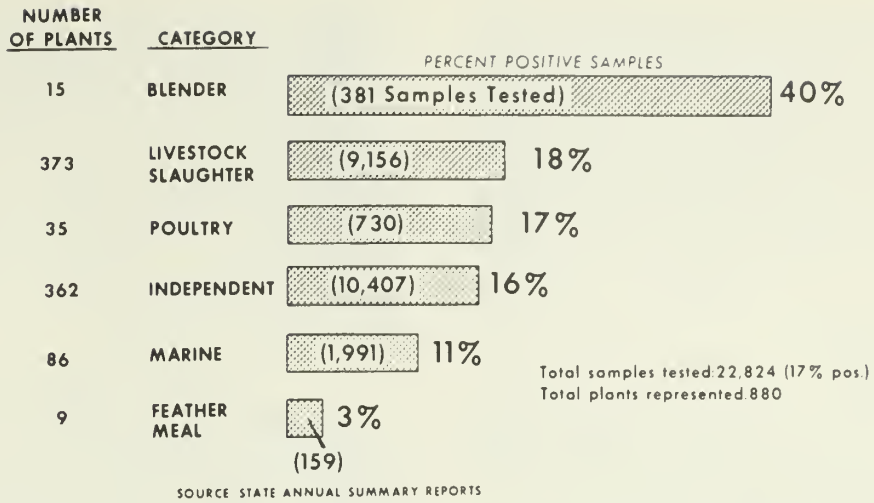

Figure 1.–Summary of test samples of finished product by category, Fiscal Year 1969.

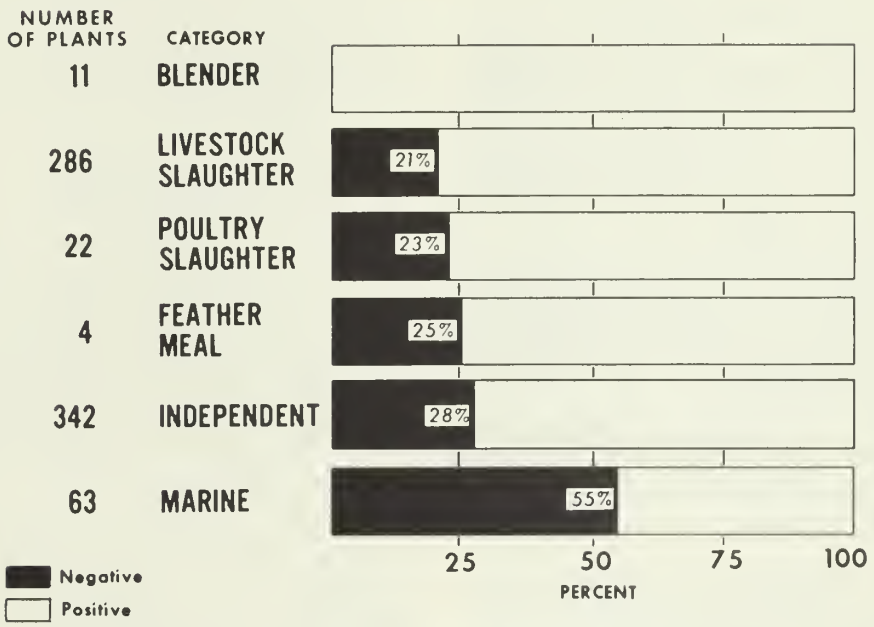

Figure 2.–Status of 728 evaluated plants at close of Fiscal Year 1969.

The evaluation phase of the program is completed. The "Clean-up Phase" of the program will be initated on a national basis in fiscal year 1971. Discussions with industry representatives regarding implementing the "Clean-up Phase" of the program and designating plants as "Approved" establishments has been completed. Last year industry representatives expressed the desire that no plants be designated as approved establishments until all plants had received a sufficient number of inspections and tests to qualify for such status.

In implementing the clean-up phase, the manager of each plant will be contacted and informed of the classification of his plant (positive or negative) and of the objectives of the other two phases of the program.

Program inspections will be discontinued in those plants that do not request continued program coverage for the purpose of achieving "Approved Status." Managers of plants requesting continued program coverage whose operations are not in compliance with the guidelines will be asked to set goals and establish a

mutually agreed timetable to bring specific operations into compliance with the guidelines.

Program progress will be measured by the ability of plants to meet their goals, the number of plants qualifying for "Approved Status," and a reduction in the annual percentage of positive finished product samples. (In fiscal year 1969, 17 percent of the 22,824 finished-product samples examined were positive.) National goals for participating plants to achieve approved status will be based upon goals set by individual plant owners.

The major program accomplishments to date may be summarized as follows:

1. Each of the 930 plants processing protein supplements of animal or marine origin for use in livestock feed is covered by the program.

2. Uniform Methods and Rules have been developed by the United States Animal Health Association for conduct of the program.

3. Standardized inspection protocols are being used by State and Federal personnel in each State.

4. Standardized laboratory methods are used in examining program samples. The procedures recommended by the Salmonella Committee of the American Association of Veterinary Laboratory Diagnosticians[4] have been adopted for program purposes by the Animal Health Division, ARS, and the United States Animal Health Association.

The standardized laboratory procedures coupled with the standard procedures for sampling finished products will permit meaningful comparisons to be made of the results obtained in different States and over different periods of time in the same State.

5. A number of our personnel have acquired considerable knowledge regarding the operations in plants processing animal and marine proteins and in evaluating the effectiveness of decontamination procedures.

6. A regional epidemiologist position has been established in each of the four administrative regions of the Animal Health Division. These positions are filled by veterinarians who have completed the requirements for the Master of Science or Master of Public Health Degree. They are strategically located to provide assistance to each State in solving problems related to moving plants through the clean-up phase to "Approved Status."

Salmonella Diagnostic Assistance

Since 1958 the Division has provided salmonella serotyping as a diagnostic assistance service to State, industry, and university-supported animal disease diagnostic laboratories. Due to the increasing number of cultures received for serotyping, laboratories requesting this service have been asked to identify the "O" antigens and group type the "H" antigens according to the Spicer-Edwards techniques before submitting them for serotyping. To assist laboratories in acquiring this capability, the Spicer-Edwards technique is taught in the Salmonella Serology Course at the National Animal Disease Laboratory. During the past 3 years 124 persons have received this training.

Salmonella Surveillance Report

The major source for the data on nonhuman isolates in the Salmonella Surveillance Report published by the National Center for Disease Control are the reports of salmonella isolates serotyped at ANH and cooperating laboratories for diagnostic purposes. The distinction between ANH and cooperating laboratories is the source of their support. ANH laboratories are supported by ANH funds. They are located at Ames, Iowa; Atlanta, Ga.; Phoenix, Ariz.; Beltsville, Md.; and Orono, Me. Cooperating laboratories are supported by State agencies or universities. They provide us with reports of their serotyping for diagnostic purposes. They also participate in the quality-assurance-check testing program we conducted for ANH laboratories. The cooperating laboratories are: Department of Animal Pathology Laboratory, University of Maine, Orono, Me.; Paige Laboratory, University of Massachusetts, Amherst, Mass.; Utah State Department of Agriculture Laboratory, Salt Lake City, Utah, and the Animal Health Laboratories of the Wisconsin Department of Agriculture, located at Barron and Madison, Wisconsin.

Cooperative Activities With the Feed Industry

Sanitation guidelines for processors of animal feeds will be published this year. These guidelines were developed with the active assistance of the Bacteriological and Technical Committee of the American Feed Manufacturers Association and the Toxigenic and Bacteriological Committee of the National Grain and Feed Association.

A study is being conducted in the Midwest to determine if the use of terminally heated meat mill will result in the elimination of salmonella from nonpelleted feeds in the mills under study.

Summary

The elimination of animal feeds as a source for the transmission of salmonella to livestock and poultry is an objective of the Cooperative State-Federal Salmonella

[4] See "Recommended Procedure for the Isolation of Salmonella Organisms. U.S. Agr. Res. Serv. ARS 91-68. 1968.

Program. The strategy for accomplishing this objective is a three-phase program operated on an individual basis. The first phase of the program has been completed. Program emphasis is on the voluntary efforts of plant owners and the industries involved in the processing of animal feeds and feed ingredients. The goal is for each participating plant to develop its own management-operated salmonella control program, and for salmonella control to become a part of the quality-assurance procedures of each plant.

We have tried to assume a support-to-industry role in our approach to the problem of salmonellosis in livestock and poultry. This is typified in serotyping as a diagnostic support service to public and private animal disease diagnostic laboratories. We welcome comments and suggestions from the industry regarding this support.

SALMONELLA AS RELATED TO THE FEED INDUSTRY

M. S. Cover[1]

The bacterial gneus Salmonella was first incriminated as a disease-producing agent in the late 19th century. Since then the relative pathogenicity of the various salmonella isolates has been continuously discussed and the presence of salmonella in the feed for most types of livestock has been investigated many times. Because salmonella species always have been associated with livestock, feed ingredients of animal origin have been a prime suspect as a source for these infectious agents. However, infections may appear without an apparent source.

The significance of the presence of salmonellae in feeds has never been clearly established. There have been reports of finding one salmonella serotype in the feed but a different type in the animal consuming that feed. Such reports certainly pose some questions regarding the significance of finding a salmonella species in feed and feedstuffs.

There have been volumes of published material which definitely establish the presence of *Salmonella* sp. in livestock feed and feed ingredients and the capability of initiating infection has been proved,*(1, 2, 3, 4, 5, 7, 10, 12, 16, 19, 20, 21, 22)*. The percentage of positive samples found is quite variable probably because of sampling size and techniques, materials, and methods used in making the analysis, differences in laboratory personnel, environment of the feed or feed ingredient before sampling, and handling of the sample itself before testing.

One of the first, if not the first, reports of finding salmonella organisms in poultry feeds was made in Poultry Science in 1955. The work was done by L. E. Erwin *(9)* at the Kansas State Agricultural Experiment Station. This investigation included 206 samples of poultry feed of which 77 samples produced salmonella-like colonies in differential media; three of these were identified as *Salmonella oranienburg* (1.4 percent); the others were reported to be *Paracolobactrum* sp. and *Proteus*.

Zindel and Bennett *(23)* indicated that during the past 30 years over 100 serotypes of salmonella have been found in finished feeds in the United States. As of February 1, 1968, these workers examined 808 samples of feed and feed ingredients; salmonella were isolated from 13, or 1.6 percent. Salmonella were not isolated from pelleted or extruded feeds. They stated that the low percentage of positive samples may be due to the small size of their samples (5 grams from 2-ton lots).

At the present time, there are a number of theories regarding the source of salmonellae infections in poultry. Some of these are:

1. Hatchery
2. Breeder flock
3. Feed (feed ingredients)
4. Vehicles and other carriers of feed and feed ingredients.
5. Environmental factors—birds, mice, rats, buildings, equipment, litter etc.

Likewise, day-old chicks and poults have been found to be infected. Thus, the cyclic nature of salmonellosis could be possible in more than one direction—parental (hen to offspring), nonparentally through the feed or feed ingredients, and horizontal transmission (environment). Since these cycles are already in motion, it is difficult to determine either a beginning or an end. Certainly, the numbers of organisms found in any one arc of this circle cannot be used to indicate their entry into a biological cycle such as this.

The main thrust of much of the research is the relationship of livestock (including birds) salmonellosis to food-borne illness in man. Processed poultry has always taken most of the criticism of this consumer-related public health problem. In my opinion, this has occurred essentially because there has been more research, diagnostic microbiology, and attention given to salmonellosis in poultry. No other consumer-related, public health-related item as salmonellosis has the same impact on the individual livestock species as *S. pullorum*. At one time, pullorum was the most important and costly disease of the industry. Today, because of the volume of fine research and by it the establishment of excellent control procedures, it is no longer a threat to

[1] Manager, Poultry Veterinary Service Division, Veterinary Department, Ralston Purina Company, St. Louis, Mo.

the industry. This control has been accomplished without direct relationship to feed contamination. The interspecies serological and cultural relationships between *S. pullorum* and *S. typhimurium* and many other salmonellae have provided more connections of the Salmonella sp. to poultry than to any other meat livestock group. I would not in any way want to either minimize nor to exaggerate the relative position of salmonellosis in the public health area. Some of the human illness caused by salmonella, particularly in the very young and in debilitated elderly people, is of a serious nature.

There is strong evidence and still more is accumulating that pelleting feeds many times eliminates salmonella from feeds or at least greatly reduces it. For example, the Ralston Purina Company in cooperation with State, federal, and local scientists have carried out turkey-feeding tests and have examined pelleted feed for the presence of salmonella. In over 700 tests of these feeds no salmonellae were found.

In a review of Crane and coworkers *(8)*, the pelleting of swine supplements made from contaminated meat meals did result in salmonella-free products. Other similar data concerning pelleted feed is available. This indicates to me that if the industry wants to take the step toward reducing the presence of salmonella there are available some techniques such as pelleting which would greatly reduce if not eliminate the organism from feeds. There would be certain added costs to such programs, which are characteristic of any new venture such as this. To be effective, of course, all feeds would have to be pelleted, including home-grown and fed rations.

Vaughn[2] has indicated that he has made attempts to discover the transmission cycles of salmonellosis in Louisiana. Such followup studies (1964-66 of approximately 125 human cases) did not discover a transmission cycle, at least none with any degree of regularity or certainty. Still, because of the number of investigations, most experts indicate that contaminated feed ingredients are the major sources of salmonella in domestic animals.

I have mentioned above the salmonella cycle, and in any discussion of the salmonella problem one must include all segments of this circle. The figure published by Nape *(18)* indicates the complexity of this problem by indicating eight loci on this circle, viz.:

a. Salmonella-infected animals
b. Other animals
c. Packing plant
d. Contaminated offal
e. Rendering plant
f. Recontaminated animal feed ingredients
g. Feed mill
h. Contaminated feed

It is of the greatest importance to include all parts of this cycle in our discussion of salmonellosis. To omit any one segment is inviting failure in any programming. The feed manufacturers certainly are an important part of the program, but only a part. Nothing can be accomplished without all the other segments (people) who constitute the full and complete problem.

The circle includes man (contaminated and contaminating man) as a contributor to the problem at two different segments.

Some reports indicate that contaminated feed plays a relatively minor role in the transmission of salmonella in chickens. Contaminated feeds can produce disease; in fact, there is evidence that one organism may cause an infection *(15)*. There is also some evidence that feeding low levels of *Salmonella typhimurium* in chickens and turkeys does not adversely affect feed consumption, weight gain, or livability even in the presence of stress.[3]

The viability of salmonella in feeds has received considerable attention in recent years. The work by Liu, Snoeyenbos, and Carlson *(14)* concerning the thermal resistance of salmonella in feed is an important contribution. This data indicates that:

1. Salmonellae populations are relatively stable in feedstuffs at moisture levels of 5 and 10 percent.
2. Salmonellae populations decline rather rapidly in feedstuffs at moisture levels of 15 and 20 and often at 25 percent.
3. Salmonellae multiply in most feedstuffs of high water activity levels.

The water activity of a product, which is closely but not absolutely related to moisture level, is the critical determinant of microbial growth. Classical salmonella growth curves characterized successively by a lag phase, rapid growth, a plateau, and rapid population decline were found in feedstuffs at or above critical water activity levels. Peak populations were reached within 3 or 4 days.

The critical water activity of a feedstuff which will allow salmonella growth appears to occur at a moisture level of approximately 30 percent (+ - 5 percent). These values probably can be narrowed when more work is completed. Normal "die off" of a salmonella population generally accelerates as water activity approaches the level that allows growth. The rate of "die off" is directly related to storage temperature.

[2] Personal communication. 1965.

[3] Rossow, C.F., and Laramore, C.R. Unpub. data. Res. Dept. Ralston Purina Co., St. Louis, Mo. 1965-66.

No clear-cut evidence has yet been found to indicate that there are marked differences among serotypes in population-moisture relationships.

The ultimate would be to produce feed free of salmonella. However, at the present time, there are information gaps and technical difficulties which prevent the industry from reaching this goal.

Snoeyenbos and coworkers *(20)* did an excellent and thorough study of the epidemiology of salmonellosis in chickens which was reported in 1967. This study included the determination of flock infection from feeding naturally contaminated feeds. Although, pathogenic salmonella have been demonstrated in feeds this is the first time a direct data had been developed to indicate the frequency of infection after the ingestion of feed containing salmonella organisms.

Liu and coworkers *(14)* have recommended a temperature of 190° F. for industrial use in making pelleted feeds. This is higher than can be now obtained by many mills. The influence of such a temperature on the quality and availability of some of the nutrients has not been clearly established. Treatments which would reduce the salmonella population by 5 logs might be considered as producing a reasonably safe feed. Assuming a salmonella flora level of 10 cells per gram, such treatment would reduce the population to one cell per 10,000 gram. The above work is presented as an example of the cooperation that now exists and has existed between industry and research in an effort to solve this problem.

There are reports calling attention to the possibility of contamination of animal byproducts and equipment from slaughter of carrier animals, from human sources, flies, rodents, dogs, pigeons, sparrows, wild birds, and feed sacks. Part of such a discussion by Morehouse and Wedman *(16)* is worthy of repeating here.

The summary of the survey by the Animal Disease and Eradication Division of the U.S. Department of Agriculture indicated that:

1. Primary attention has been focused on salmonella. Isolation of a limited number of other pathogens from animal byproducts has been reported, but their disease significance has not been determined.
2. Definitive evidence that animal byproducts in rations are sources of causative organisms responsible for specific field occurrences of salmonellosis is lacking. The potential disease threat posed by these organisms in animal byproducts is worthy of further analysis.
3. Recontamination of animal byproducts after their processing is believed the principal factor accounting for the presence of Salmonella sp in them; possible sources of this contamination are rodents, wild birds, dogs, and human handlers of products.
4. The pathogenicity of various salmonella serotypes and the ability of these organisms to induce disease or carrier states when introduced in poultry or animal rations seems worthy of further study.
5. Industry groups involved in this problem are conducting an active research program designed to investigate procedures and environmental problems associated with possible contamination of some products. They are willing to place into effect whatever measures are necessary as such methods are proved effective.

Since feed has been shown to contain salmonella, then there are a number of important related questions regarding this part of the epizootiology.

1. How many organisms per unit of feed or feed ingredient are required to produce infection?
2. How long do they or can they live in the feed?
3. Do salmonella multiply in the feed or in feed ingredients or do they tend to gradually reduce in number with storage?
4. What environmental factors influence this livability; such as pH, moisture, temperature, temperature changes?
5. Are certain ingredients harmful to the salmonella? Are others beneficial to salmonella livability?

The subject of the presence of Salmonella sp. in feeds and feed ingredients has received considerable attention since 1955. More recent work *(6, 11, 13, 17)* is just beginning to explore the effect of temperature, chemical composition, physical characteristics, water activity, and other similar factors on the livability of salmonella in feeds. Certainly the presence of the organism has been established. We now need to determine the position on the cycle when it can best be broken. Also, it is apparent that there is more than one cycle of infectivity as has been mentioned previously. The livestock industry (including poultry) has been active in stimulating and supporting research to illuminate some of these areas. There are numerous examples of this.

For one, the work by Liu *(14)*was supported by the American Feed Manufacturers Association. There is a need for continued research activity to build upon the new knowledge, to sustain the improvements in technique, and to persist in finding better feed production techniques. The contamination of feed ingredients has been widely studied. There is excellent cooperation between scientists, regulatory officials, and the rendering industry in establishing the cause and cure for the presence of salmonella in rendered products. Recent revision of the manufacturing guidelines for this industry are

definite indications of the desire of this industry to solve this problem.

In summary, I want to reemphasize the scope of the problem of salmonellosis and the many ways that salmonella enter and may continue in the cycle. We cannot make progress in any one part without cooperation with all of the others. Secondly, the feed industry and the feed ingredient industry have been and will continue to cooperate fully with others involved in this problem to bring about a solution. Thirdly, these two industries have been and are still at work intramurally to monitor themselves in order to find ways for improving their final product. This problem will be solved as we continue to cooperate by doing research that finds ways to improve production techniques that are consistent with the economics of the industry.

LITERATURE CITED

(1) Bayne, H. G., Garibaldi, J. A., and Lineweaver, Hans. Heat Resistance of *Salmonella typhimurium* and *Salmonella senftenberg* 775-W in Chicken Meat. Poultry Sci. 44: 1281-1284. 1965.

(2) Bierer, B. W., and Eleazer, T. H. Clinical Salmonellosis Accidentally Induced by Feed and Water Deprivation of One Week Old Broiler Chicks. Poultry Sci. 44: 1606-1607. 1965.

(3) Boyer, C. I., Brunner, D. W., and Brown, J. A. Salmonella Organisms Isolated From Poultry Feed. Avian Dis. 2: 396-401. 1958.

(4) Narotosky S., Brunner, D. W., and Brown, J. A. Salmonellosis in Turkeys and Chickens Associated With Contaminated Feed. Avian Dis. 6: 43-50. 1962.

(5) Burr, W. E. and Helmbolt, C. F. Salmonella Species Contaminants in Three Animal By-products. Avian Dis. 6: 441-443. 1962.

(6) Calhoun, C. L., and Frazier, W. C. Effect of Available Water on Thermal Resistance of Three Nonsporeforming Species of Bacteria. Appl. Microbiol. 14: 416-420. 1966.

(7) Clise, J. D., and Swecker, E. E. Salmonella From Animal By-products. Public Health Rpts. 80: 899-905. 1965.

(8) Crane, F. M., Hanser, M. and Schagel, L. Salmonella in Feedstuffs. Feedstuffs, July 8, 1967.

(9) Erwin, L. E. Examination of Prepared Poultry Feeds for the Presence of Salmonella and Other Enteric Organisms. Poultry Sci. 34: 215-216. 1955.

(10) Griffin, C. A. A Study of Prepared Feeds in Relation to Salmonella Infection in Laboratory Animals. Jour. Amer. Vet. Med. Assoc. 121: 197-200. 1952.

(11) Hansen, H. H. and Riedmann, H. Factors Affecting The Heat Resistance of Nonsporing Bacteria. Jour. Appl. Microbiol. 26: 314-333. 1963.

(12) Hussemann, D. L., and Buyske, J.K. Thermal Death Time - Temperature Relationships of *Salmonella typhimurium* in Chick Muscle.

(13) Kowalsk, L M., and Stephens, J. F. Persistance of Arizona Paracolon 7: 1, 7, 8 in Feed and Water. Poultry Sc. 46: 1349-1660. 1967.

(14) Liu, T. S., Snoeyenbos, G. H., and Carlson, V. L. Thermal Resistance of *Salmonella senftenberg* 775W in Dry Animal Feeds. Avian Dis. 13: 611-631. 1969.

(15) Milner, K. C., and Shaffer, M. F. Bacteriologic Studies of Experimental Salmonella Infections in Chicks. Jour. Infect. Dis. 30: 81-96. 1952.

(16) Morehouse, L. G., and Wedman, E. E. Salmonella and Other Disease Producing Organisms in Animal By-products. A Survey. J. Amer. Vet. Med. Assoc. 139: 939-995. 1961.

(17) Mossel, D. A., and Koopman, M. J. Losses in Viable Cells of Salmonella Upon Inoculation Into Dry Animal Feeds of Various Types. Poultry Sc. 44: 890-893. 1965.

(18) Nape, W. F. Recovery of Salmonella From Material in Feed Mills. 72nd Ann. Meeting USLSA Proc., New Orleans, La. 1968.

(19) Rasmussen, O. G., Hansen, R., Jacobs, N. J., and Wilder, O. H. M. Dry Heat Resistance of Salmonellae in Rendered Animal By-products. Poultry Sci. 43: 1151-1157. 1964.

(20) Snoeyenbos, G. H., Carlson, V. L., McKie, B. A., and Smyser, C. F. An Epidemiological Study of Salmonellosis of Chickens. Avian Dis. 11: 653-667. 1967.

(21) Smyser, C. F., Bacharz, J., and Van Roekel, H. Detection of *Salmonella typhimurium* From Artificially Contaminated Poultry Feed and Animal By-products. Avian Dis. 7: 423-434. 1963.

(22) Watkins, S R., Flowers, A. I., and Grunbles, I. C. Salmonella Organisms in Animal Products Used in Poultry Feeds. Avian Dis. 3: 290-301. 1959.

(23) Zindel, H.C., and Bennett, M. C. Salmonella Organisms in Poultry Feeds. Poultry Sci. 47: 1925-1927. 1968.

FINISHED PRODUCT CONTAMINATION SUCH AS FEED, EGGS, AND READY-TO-EAT ITEMS FROM THE FOOD AND DRUG STANDPOINT

James E. Porter[1]

I have been asked to briefly discuss salmonella contamination of finished products such as feed, eggs, and ready-to-eat items from the Food and Drug standpoint. First I'd like to call your attention to a few definitions that are contained in the Federal Food, Drug, and Cosmetic Act.

The term "food" means articles used for food or drink for man or other animals, and articles used for components of any such article.

The term "animal feed" means an article which is intended for use for food for animals other than man and which is intended for use as a substantial source of nutrients in the diet of the animal, and is not limited to a mixture intended to be the sole ration of the animal.

Adulterated Food

A food shall be deemed to be adulterated, if it bears or contains any poisonous or deleterious substance which may render it injurious to health, and if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food; if it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health; or if it is in whole or in part the product of a diseased animal or an animal which has died otherwise then by slaughter.

Incidence of Salmonella Contamination

Samples of domestic animal byproducts examined by FDA. Ten subsamples per sample examined. This period covers July 1, 1969, thru April 30, 1970. There were 810 samples, including subsamples, examined. The distribution pattern is as follows:

8 samples showed 10 of 10 positive
7 samples showed 9 of 10 positive
2 samples showed 8 of 10 positive
4 samples showed 7 of 10 positive
4 samples showed 6 of 10 positive
6 samples showed 5 of 10 positive
4 samples showed 4 of 10 positive
7 samples showed 3 of 10 positive
6 samples showed 2 of 10 positive
5 samples showed 1 of 10 positive
28 samples showed 10 of 10 negative

FDA Testing of Imported Lots of Fish Meal

A total of 94 lots were examined with 14 detentions. A total of 29 lots of imported tankage and bonemeal were examined with 4 lots detained.

Salmonella examinations were made by FDA laboratories for all product categories, such as, dry, frozen, and fresh broken eggs, dry milk and other dairy products, dry yeast, miscellaneous foods, turtle meat, frog legs, pet foods, animal byproducts, domestic and imported meat scrap, fishmeal and meat products, animal food supplements, and food supplements in animal feed. During fiscal year 1969, 4,414 samples were examined with 336 samples salmonella positive. The number of samples examined during the first three-quarters of fiscal year 1970 totaled 3,031 with 250 samples found salmonella positive.

Salmonella Research

A joint research project for studying the transfer of resistance of salmonella organisms and the resistance to certain antibiotics between micro-organisms is under way between FDA and the Animal Health Division, Agricultural Research Service, USDA and the Public Health Service, National Center for Disease Control. Several other minor research projects have also been conducted during the past fiscal year.

Education

The Food and Drug Administration has undertaken an educational program, aimed principally at the consuming public and the producer of feeds and feedstuffs. Such an education program consists of the dissemination of fact sheets, pamphlets, news releases, radio and TV announcements and the conducting of seminars and workshops, as well as presentation of

[1] Food and Drug Administration, Washington, D.C.

scientific papers before consumer groups, industry groups, and the academic community.

Regulatory Actions

The Food and Drug Administration has certain regulatory actions that can be invoked against offenders, such as citation, seizure, detention prosecution both civil and criminal, and injunction.

Cooperative Salmonella Program

I don't think I should speak too much about this point, since Dr. Saul Wilson of the USDA Animal Health Division has described the working relationship between their agency and ours. This program has worked very well in the past and I hope it will continue to do so in the future. Phase 2 of the program is scheduled to commence July 1, 1970.

MEASUREMENT AND CONTROL OF EGG SHELL CONTAMINATION

R. F. Gentry[1]

The objective of any sanitation program dealing with production of table or hatching eggs is the development of procedures to minimize the contamination of either the eggshell or the interior of the egg. Work at Penn State has been concerned primarily with development and use of methods for detection of eggshell contamination and the evaluation of some management practices that might reduce this contamination. We have been primarily considering the total number of bacteria on the shell rather than identification of specific species. Since you cannot selectively control certain bacteria in the environment, it is necessary to develop procedures which will significantly reduce or eliminate the total bacterial flora.

The first thing to consider is possible sources of contamination. In any poultry house there is a limited number of sources of contamination. The primary ones are feed, water, air, and litter. The amount of contamination varies greatly depending upon the temperature, humidity, and various management factors. However, in general, they can be characterized as follows:

Bacteria from natural sources

Source	*Level*	*Control*
Feed	Low	Pellets
Water	Low	Sanitizers
Air	Low or high	Litter management
Litter	High	? Chemical - Wire

Considering feed as average to good quality, the bacterial contamination is, in most cases, relatively low. This can be further reduced by procedures such as pelleting but is seldom a significant problem by itself. If waterers are cleaned routinely and a sanitizer employed, levels of water contamination can be kept relatively low.

Air contamination is dependent on the type and condition of the litter. If litter is damp or relatively clean without a great amount of dust, then the bacterial count of the air will be low. If litter is dry with large amounts of dust, then counts will be proportionately high. If litter could be eliminated completely, then only the droppings would be available as a cource of contamination. Also, if the birds were not in contact with the droppings, they could then accumulate and a small amount of dust would be raised. This is obvious in cage operations where the total dust is always less than in litter houses.

Litter contamination can be reduced by the application of a chemical to kill the bacteria, such as Vineland's "Methogen." Our approach was removal of the birds from the litter by placing them on wire floors.

The wire floor houses had sloping wire floors with rollaway nests. These wire floor houses were compared with conventional litter floor houses with litter nests. The following tabulation shows the results of the comparisons.

Comparison of wire versus litter floor–pen 3

	Litter	*Wire*
Bacteria per cubic foot of air	13,000	600
Coliforms per cubic foot of air	1,100	160
Positive for coliforms:	*Percent*	*Percent*
Late dead and pipped embryos	94	33
Cull chicks	100	20
Normal chicks	73	35

An Andersen Air Sampler was used to determine the bacterial contamination. The litter floor house averaged 13,000 bacteria per cubic foot compared with only 600 in the wire floor house. Also of significance is the great decrease in coliform counts, which were 1,100 per cubic foot of air in litter floor houses compared with only 160 in the wire floor house. Eggs from these two houses were incubated and hatched separately. At 1 day of age, the late deads and pipped embryos, cull chicks, and normal chicks were cultured for presence of coliforms in the yolk or intestine or in both. Ninety-four percent of the late dead and pipped embryos from litter floor house were positive compared with 33 percent from the wire

[1] The Pennsylvania State University, University Park, Pa.

floor house. Cull chicks from the litter floor house were all positive compared with 20 percent from the wire floor house. In the normal chicks 73 percent from the litter floor house contained coliforms compared with 35 percent from the wire floor house. The amount of contamination within the day-old chick has been consistently much higher in chicks from litter floor houses. We believe the source of this increased infection in the chicks is due to eggshell contamination which is picked up at the time the chicks are hatched. Since we used clean eggs, free of gross fecal material, it was not likely that contamination resulted from shell penetration. It is most likely that the chicks become contaminated by exposure of the raw umbilicus at the time of hatching to organisms that remained on the eggshells. A newly hatched chick that comes in direct contact with contaminated shells can develop the infection within a matter of hours. Additional birds have shown a consistent correlation between the number of infected chicks and the amount of bacterial contamination in the air.

In another set of houses, similar results were obtained for the amount of air contamination. In this case, fungi per cubic foot of air were also determined. Although we have routinely found great differences in the number of bacteria in the air, the number of fungi has not been significantly different. This may be due to the technique used with the Andersen Air Sampler. However, tests with Saborauds media have failed to improve the recovery of fungi. Plates used in the Andersen Air Sampler are filled with tryptose agar, which we found to be the best single media for both bacteria and fungi. Since there were such marked differences in the air contamination, the possibility of eggshell contamination was investigated. In this case, the average total number of bacteria on the shell was 90,000 with eggs from the litter house compared with only 2,400 on eggs from wire floor house (see following tabulation).

Comparison of wire versus litter floor–pen 2

	Litter	*Wire*
Bacteria per cubic foot of air	9,500	1,100
Fungi per cubic foot of air	200	120
Bacteria on eggshell	90,000	2,400

All of these values are the average for six eggs.

The practicality of using wire floors for breeding birds was investigated on these same flocks, since there had been questions whether or not birds could breed properly because of the instability of the floors. The total production, fertility, and hatchability over a 9-month period is shown in the following tabulation.

Comparison of wire versus litter floor–pen 1

	Litter Percent	*Wire Percent*
Production	73	72
Fertility	96.5	96.2
Hatchability	92.3	94.2

Production and fertility are virtually the same on both the wire and litter floor houses. It is interesting to note that hatchability was approximately two percent greater from wire floor eggs. This increased hatchability has consistently averaged from 2 to 4 percent.

In order to measure the affect of various management procedures on the degree of eggshell contamination, a method for culturing the total bacteria on the shell was developed.

Sterile plastic bags measuring 6 x 10 inches were employed. Each bag received 10 ml. of sterile buffered saline, and the egg then dropped into the bag. All eggs were first wiped clean with a sterile paper towel to remove any gross fecal material. The surface of the egg was rubbed through the bag for 1 minute, a metal bag tie fastened around the top so the solution would completely cover the egg which was allowed to soak for 5 minutes. The tie was then removed and the egg rubbed for another minute and pushed out through the top of the bag. The total bacterial count was determined by titering this egg washing, using tryptose agar pour plates. After 24 hours incubation, plates were read and the total number of bacteria from the entire eggshell was calculated.

The general procedure used for making an eggshell contamination count was as follows:

- Plastic bags (6 x 10 inches) were sterilized in a gas sterilizer.
- The eggs to be tested were collected in new, clean polystyrene containers and any surface material wiped off with a sterile towel.
- The egg was dropped into the plastic bag containing 10 ml. of sterile buffer.
- The top of the bag was held shut and the egg rubbed through the plastic for 1 minute.
- The egg was then put into one corner of the bag and a wire tie used to fasten the bag around the top so the egg was completely encased in buffer solution. After 5 minutes soaking, the tie was removed, the egg again rubbed for 1 minute, and the egg pushed out through the top of the bag.
- One ml. of the wash solution was then removed and considered as the 10^{-1} (1 to 10) dilution for the titration since a total of 10 ml. was used for the washing. The 1.0 ml. was placed directly into a sterile petri dish.
- An additional 0.2 ml. was removed and mixed with

1.8 ml. of buffer, then titrated (tenfold dilutions) through 10^{-5} dilution.

- One ml. of each dilution was then transferred to a plastic petri dish.
- Tryptose agar was then added to the plates and the plates swirled.
- After incubation for 24 hours at 37° C. the plates were then counted and the total bacteria calculated.

This method has given highly reproducible results. The counts of eggshell bacteria correlates with the gross contamination in the air and is considered a reliable method for measuring total eggshell contamination.

In order to obtain basic information concerning eggshell contamination, eggs were selected from the collection belt of a large, wire floor house and classified on gross appearance as clean, soiled, or dirty. Eggs that showed no evidence of any discoloration were classified as clean. When faint lines were evident where the egg had rolled over dirty wire or onto a dirty spot on the collection belt, they were classified as soiled. Only those which had gross contamination with fecal material were classified as dirty. In all cases, clean, soft, sterile, paper towels were used to gently wipe the surface of the egg to remove any particulate material. We found it necessary to remove all traces of fecal material since fresh feces gave counts averaging 2 to 6 billion bacteria per gram. Therefore, even the slightest amount of feces on the eggshell would give an extremely high count. Nine eggs per group were tested. Clean eggs averaged only 3,400 bacteria, soiled eggs near 26,000 and dirty eggs 400,000 (see following tabulation).

For tests on the effect of storage, incubation, and treatment with sanitizers, eggs classified as soiled were used. Clean eggs have very low counts and the numbers are not sufficient to permit a meaningful evaluation. One of the first trials conducted was a measure of the effect of incubation in a forced-air egg incubator. Results are shown in table 1.

Bacterial counts from eggs

Gross classification	Total bacterial counts		
	High	Low	Average
Clean	6,400	200	3,400
Soiled	57,000	11,000	25,700
Dirty	930,000	110,000	390,000

Average of 9 determinations per group.

It was surprising to learn that the contamination on the eggshells decreased throughout the 21 days of incubation. Each determination represents the average of counts from six eggs. When first placed in incubator at 0 days, untreated control eggs averaged 36,000. By 11 days the counts had dropped to 200 and remained relatively constant to 21 days incubation. In an attempt to determine if there was an accumulation of bacteria on the shell during incubation, eggs were first washed with 70 percent alcohol. It is obvious that even in this case, there is a gradual decrease of bacteria on the shell and no evidence of bacterial contamination from the air of the incubator. Eggs that had been previously dipped in 200 p.p.m. quaternary ammonia (QA) remained completely negative for bacteria throughout 21 days. Each value listed for "daily control" is the average of six eggs. These eggs were washed by the routine procedure for determining total counts. An additional six eggs collected at the same time were washed in the same bags that had been used for the six eggs from the 200 p.p.m. quaternary ammonia tests. In other words, when the test on the eggs with 200 p.p.m. QA had been completed, eggs collected fresh on that day were placed in the bag and washed in the same buffer. If any quaternary ammonia had remained on the incubated test eggs, it would then be in the buffer and might affect the counts when the same buffer was used to wash the untreated

Table 1.—Levels of contamination during incubation

[Each value is the average for 6 eggs]

Treatment	Bacterial counts after indicated days in forced-air egg incubator						
	0	3	7	11	15	18	21
Untreated control	36,200	17,600	3,200	200	90	250	80
Alcohol washed	250	140	70	150	30	10	3
Dipped quaternary ammonia 200 p.p.m.	0	0	0	0	0	0	0
Daily control	—	33,300	30,800	18,700	33,200	11,000	20,100
Daily eggs washed in residue from quaternary ammonia eggs	—	0	0	5	0	0	0

daily controls. Sufficient quaternary ammonia remained on the shells through 21 days incubation to completely kill off the bacteria which would have been equivalent to the daily controls. This is significant since the bacteria were not only eliminated by the quaternary ammonia but a residue remained to kill off any bacteria that might accidentally come in contact with the eggshell.

The use of quaternary ammonia was based on previous trials comparing various disinfectants for spraying eggs (table 2). Eggs were sprayed with chlorine dioxide, hypochlorite, iodine, two different quaternary ammonia products, and water. The average counts at the time of spraying were 41,500. After 24 and 48 hours at egg-cooler temperature (approximately 65° F.) samples of five eggs were removed and total counts made. The chlorine dioxide had little affect on the total bacteria. When compared with spraying with water, it would be considered to have less than no value.

Hypochlorite greatly reduced the numbers but iodine failed to be effective. Most quaternary ammonias completely eliminated all bacteria.

Table 2.–Comparison of disinfectants for spraying eggs

[Each value is average for 5 eggs]

Disinfectan spray	Bacterial count after–		
	0 hours	24 hours	48 hours
Control	41,500	—	—
Chlorine dioxide (80 p.p.m.)	—	31,000	24,200
Hypochlorite (200 p.p.m.)	—	420	190
Iodine (100 p.p.m.)	—	19,100	8,200
Quaternary ammonia No. 1 (200 p.p.m.)	—	<10	<10
Quaternary ammonia No. 2 (200 p.p.m.)	—	<10	<10
Water	—	23,400	5,000

In another experiment with quaternary ammonia at various concentrations (table 3), there was a marked reduction in total bacterial counts at concentrations as low as 50 p.p.m. Since there was a complete kill of bacteria at 200 p.p.m., this was the concentration selected for routine use.

As a result of these findings, it is our conclusion that quaternary ammonia compounds are the product of choice for spraying or dipping of eggs to control shell contamination. The residual activity will prevent the subsequent recontamination of the egg as well as supplying a clean medium when chicks are hatched.

It is obvious that the use of wire floors will greatly reduce shell contamination but cannot completely eliminate it as a possible problem.

A diagrammatical representation of what we think is the method that contamination can pass from one flock to another by way of the egg and the chick follows.

Sources of *E. coli* and bacterial contamination

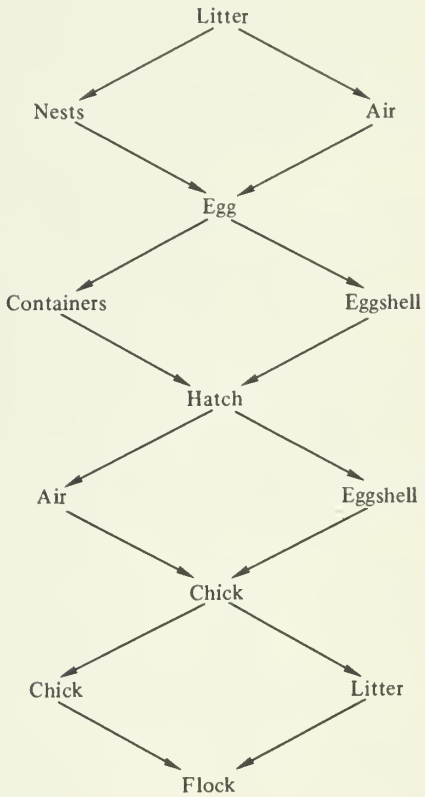

Table 3.–Effect of quaternary ammonia on eggshell contamination during incubation

[Each value is average for 10 eggs]

Treatment	Bacterial counts after indicated days in forced-air incubator						
	0	3	7	11	15	18	21
Untreated control	14,600	11,200	6,600	4,200	1,600	180	420
Dipped QA 50 p.p.m.	—	0	30	0	4	12	—
Dipped QA 100 p.p.m.	—	0	0	0	6	12	24
Dipped QA 200 p.p.m.	—	0	0	0	0	0	0

Litter and droppings carry bacteria and from this the nests and air are contaminated. These serve as sources of contamination for the eggshells and the containers in which the eggs are collected. During hatching, bacteria are given off into the air and spread through the hatcher. More significant, however, is the possible contamination of the umbilicus when it comes in contact with a contaminated shell. Once a chick is contaminated, it can transmit infection directly to other chicks or indirectly into the litter through the feces. In either case, the same bacterial floria will be carried into the new flock.

Although none of this is directly associated with salmonella, it is obvious that control of the total bacterial population is necessary to reduce or eliminate salmonella contamination. Production of healthy chicks requires that a contamination control program start in the breeding house. This can be aided by improved management and housing practices. However, eggshell contamination is the link that can and should be broken. This can be accomplished by proper sanitizing of the eggs.

THE SCREENING OF DRUGS FOR THE POTENTIAL ERADICATION OF GRAM-NEGATIVE BACTERIA (SALMONELLA AND PARACOLON) FROM HATCHING EGGS

Julius Fabricant[1]

Salmonella and paracolon organisms are widely distributed and important contaminants of hatching eggs in chickens, turkeys, and ducks. As a consequence of such egg contamination these infections are transmitted to the day-old chicks, poults, and ducklings, and are occasionally accompanied by disease and mortality in these infected birds. Further losses have occurred due to rejection of shipments of birds when such infections have been detected by cultural procedures. In addition, the frequent presence of salmonella in poultry is considered a constant threat to human health by many public health officials.

Up to this time no procedure has succeeded in completely eliminating these organisms from breeding birds, although serological procedures, egg fumigation, and chick or poult treatment have decreased the levels of infection. Since we have succeeded in a complete eradication program for *Mycoplasma gallisepticum* by temperature differential egg dipping, we feel that such a process of removing gram-negative infections from both the surface and interior of hatching eggs is a necessary step in the eradication of these agents as poultry disease problems.

Several factors suggest that the use of such a product would be economically feasible in terms of marketing potential. First, it is highly likely because of the relatively great danger of reinfection that such a process would not be limited to primary breeders but would be used by many parent breeder flocks as well. Secondly, since the product will not be used on eggs or birds that are destined for human consumption, it may be possible to avoid some of the expensive tests necessary for normal drug clearance.

We have devised a procedure for producing an almost 100 percent infection rate in hatching eggs with *Salmonella typhimurium.* This organism was selected because it is the most widely distributed salmonella in poultry, because it is an undoubted pathogen, and because it is one of the most resistant gram-negative organisms to therapy. Any drug that would remove *S. typhimurium* is highly likely to remove all gram-negative rods.

Our screening procedure would be to infect groups of 40 eggs with *S. typhimurium* and then to treat an infected group of eggs with each of three suggested levels of drug solution. The treatment will be by dipping eggs warmed to 37° C. into drug solution at 4° for 15 minutes. This is essentially the procedure that was successful in producing levels of tylan in eggs sufficient to eliminate *Mycoplasma gallisepticum.* Other comparable groups of uninfected eggs would be similarly treated to insure that the treatments used had no deleterious effects on hatchability.

All infected, treated eggs as well as the infected, untreated controls would be cultured for *S. typhimurium* after 18 days of incubation thus allowing the chick embryos to act as an enrichment medium for the detection of minimal rates of infection. The effectiveness of the drug treatment would therefore be measured by its ability to remove all detectable salmonella infection from the chick embryos (see table 1).

Table 1.–Protocol of drug screening procedure

Therapeutic activity		
All eggs infected:		
Group		
1 Drug level 1	40 eggs	all cultured for 18 days
2 Drug level 2	do.	do.
3 Drug level 3	do.	do.
4 Untreated controls	do.	do.
Effect on hatchability		
All eggs uninfected:		
5 Drug level 1	40 eggs	all eggs hatched
6 Drug level 2	do.	do.
7 Drug level 3	do.	do.
8 Controls	do.	do.

Any drugs that proved satisfactory on the basis of such primary screening would be investigated further under conditions designed to simulate the natural pattern of infection and then tested under field conditions.

The drugs to be screened should be:

1. Soluble
2. Stable at 37° C.
3. Active against *Salmonella typhimurium*

[1] Department of Avian Diseases, New York State Veterinary College, Cornell University, Ithaca, N.Y.

FACTORS IN HATCHING EGG SANITATION

J. E. Williams[1]

Adult intestinal carriers serve as the chief source of the motile salmonellae in most species of poultry. Fecel contamination of the hatching eggs by the chronic carrier or from an infected environment is the means by which the infections are generally introduced into the incubator and subsequently into the brooder.

We are beginning to recognize that it is in relation to the production of eggs free of shell contamination with salmonella organisms that our most effective means of controlling salmonella infections exists. Attention to the egg at the supply flock level and subsequently at the level of the incubator and the hatchery offers the means of breaking the natural transmission cycle of the infections. The use of salmonella-free eggs from salmonella-free flocks will greatly lessen chances of introducing the infections into the incubator through fecal contamination. This cannot be a partial program but must include all supply flocks and all eggs coming into the hatchery.

At the supply flock level, adequate number of nests should be provided. Eggs should be collected at frequent intervals, four or five times a day, routinely fumigated as soon as possible after collection, and stored in a cool place for as short a period as possible before setting. There is a growing interest in the use of wire floors and rollaway nests for hatching-egg flocks. Cleaned and disinfected containers should be used in collecting the eggs, and the person making the collections should be certain that he does not serve as a source of contamination from organisms that may be present on his clothing or hands. Dirty eggs should not be used for hatching purposes, and should be collected in a separate container from hatching eggs.

It is best to remove dirt and dried feces from eggs by dry sanding. Egg dipping and spraying procedures have been proposed and are presently in use for the destruction of salmonellae on the surface of both hatching and market eggs prior to the penetration of the organisms through the shell and shell membranes. To be effective such procedures must be properly applied and done immediately after egg collection. Wash solutions can become excessively contaminated with organic matter and bacteria to the extent that dipping practices can do more harm than good. The same is true of sprays that damage the natural protective qualities of the eggshell and can serve as the means of transporting organisms through the eggshell.

Early on-the-farm preincubation fumigation of hatching eggs with formaldehyde gas has been found to be very effective in the prevention of egg-borne paratyploid infections. Early fumigation is essential since there exists no means of destroying salmonella organisms once they have penetrated through the eggshell. Eggs should be fumigated within 2 hours of being laid and should be placed on racks that will permit good air circulation in a tightly-closed cabinet. Formaldehyde gas is provided by mixing 0.6 g. of potassium permanganate ($KMn0_4$) with 1.2 ml. of formalin (40 percent) for each cubic foot in the cabinet. The chemicals should be mixed in an earthenware or enamelware container having a capacity of at least 10 times the volume of the total ingredients. The gas should be circulated within the enclosure for 20 minutes then expelled. Formaldehyde gas as a fumigant gas can also be generated by heating paraformaldehyde powder.

[1] Southeast Poultry Research Laboratory, Athens, Ga.

A 100 PERCENT SEROLOGICAL TESTING PROGRAM VERSUS A MONITORING PROGRAM FOR SALMONELLA INFECTION IN TURKEY BREEDING FLOCKS

M.C. Kumar, H.R. Olson, L.T. Ausherman, W.B. Therber, M. Field,
W.H. Hohlstein, M. York, and B.S. Pomeroy[1]

Under the present Minnesota salmonella testing program all turkey breeder flocks in the State are 100 percent serologically examined for reactions against polyvalent salmonella antigen. Any reactors resulting therefrom are then screened individually against Typhimurium and Pullorum antigens. All reactors to these two antigens are taken out of the flocks and representative sample of birds is submitted to the laboratory for cultural examination. If an isolation of groups 'B' or 'D' salmonella is made, then that flock is called a reactor flock and has to be retested clean once after 21 days. Flocks where no isolation of salmonella is made and no reactors are found initially are called negative flocks.

To avoid testing 100 percent of the birds and reduce the expenses incurred by the breeder flock owners a pilot study was initiated to find a better or an equivalent test in efficiency to the 100 percent testing program.

In this study it was decided to take 500 cloacal swabs (pooled five to a tube) and conduct serological tests on 500 blood samples and compare the results with 100 percent serological test. This study involved 34 breeder flocks and the results are as follows:

Total number of flocks tested	34
Number of flocks found positive by both tests	15
Number of flocks found positive by 100 percent serological test	8
Number of flocks found positive by 500 blood tests and 500 cloacal swabs	12
Number of flocks found positive by 500 blood tests only	5
Number of flocks found positive by 500 cloacal swabs	1

The above results indicated that 500 blood tests culturing of 500 cloacal swabs is better or equival 100 percent serological testing in efficiency.

[1] University of Minnesota, St. Paul, Mo.

APPENDIX

RECOMMENDATIONS FOR THE CONTROL OF SALMONELLOSIS IN CHICKENS

The following is a general outline for the control of salmonellosis (excluding *S. pullorum* and *S. gallinarum*) in chickens. It is not intended to give specific recommendations in all areas of concern since a great deal of research work remains to be accomplished. Sources for the introduction of salmonella into chicken flocks are generally considered to be breeder flock and hatchery, environment, and feedstuffs. For the effective reduction of the problem of salmonellosis in chickens, all areas have to be taken into consideration in establishing a control program.

A. Breeder Flock and Hatchery
   1. Monitoring of Hatching-Egg Flocks
      a. A system should be established and applied to detect the presence of salmonellae. Floor or nest litter sampling or cloacal swabbing are suggested monitoring procedures. Serological testing may be useful under some circumstances.
      b. The intensity of sampling will have to be determined for each individual operation depending on the desired results and support available from monitoring programs.
   2. Hatching-Egg Sanitation
      a. Frequent collection of clean hatching eggs from clean nests.
      b. Clean hatching eggs should receive preincubation treatment as soon as possible after collection. Fumigation with formaldehyde gas or spraying or dipping with an effective disinfectant at the farm level will reduce the number of viable organisms on the surface of the shell.
   3. Hatchery Sanitation
      a. In addition to general hatchery sanitation, procedures should be taken to hatch eggs according to flock source to prevent cross infection during the hatching process. Each chick source should be kept separate during sexing, grading, or other hatchery procedures to reduce cross contamination.
      b. Individual hatcher exhaust ventilation systems will reduce cross contamination within the hatcheries.
      c. A monitoring system for salmonellae can be used to detect the possibility of environmental sources of contamination within the hatchery.

B. Environment (Except Feedstuffs)
   1. Use only confinement-type housing so constructed that it can be thoroughly cleaned and disinfected.
   2. Decontaminate all equipment used within the buildings.

C. Feedstuffs
   1. Encourage the Feed Industry and the U.S.D.A., Animal Health Division to continue their activities which are directed toward the ultimate objective of elimination of salmonellae in feedstuffs and complete feeds.
   2. The use of terminal heat treatment in the final complete feeds has been shown to effectively reduct the number of viable salmonellae provided that proper temperature, moisture, and time are used.

D. Other Considerations
   1. Quarantine Procedures
      a. Prevent access to the building of wild birds and animals (including domestic pets).
      b. Limit the movement of personnel and encourage the disinfection of foot gear.
   2. Medications
      Medications may be useful to prevent clinical salmonellosis. Available drugs do not eliminate flock infection and may interfere with detecting infection in the monitoring of flocks.
   3. Processing Plants
      a. Monitoring systems are encouraged to trace the sources of contamination and recontamination with salmonellae.
      b. In-plant chlorination procedures of washing and chilling water have been demonstrated to be of value in reducing recontamination within poultry processing plants.

RECOMMENDATIONS FOR THE CONTROL OF SALMONELLOSIS AND ARIZONOSIS IN TURKEYS

The following is a general outline for the control of salmonellosis and arizonosis (excluding *S. pullorum* and S. gallinarum) in turkeys. In order to effectively accomplish the reduction of the incidence of salmonellosis and arizonosis, the thrust must be directed at three general sources:

A. Breeder flock and hatchery
B. Environment of breeder flocks and market flocks
C. Feedstuffs

A. Breeder Flock and Hatchery

1. Breeder Flock

Objective: Establishment of primary and multiplier breeders free of salmonella and arizona infections.

a. Complete confinement of primary and multiplier breeder flocks is desirable and is recommended.

b. Determination of present status of breeder flocks

i. *Sampling of environment.*—Litter, dust, nest samples collected and examined for salmonellas and arizonas.

ii. *Sampling of Breeder flocks.*—Serological testing - Use of individual or combination of salmonella antigens (*S. pullorum* and *S. typhimurium*). Cloacal swabs - 500 samples per flock.

If a breeder flock is found infected with *S. typhimurium* it is not retained as a breeder flock except when a valuable genetic strain is involved. Attention will be given to other salmonella serotypes and arizonas as replacement breeders are selected.

c. Hatching-egg sanitation

i. Frequent collection of clean hatching eggs from clean nests.

ii. Clean hatching eggs should receive preincubation treatment as soon as possible after collection. Fumigation with formaldehyde gas, or spraying or dipping with an effective disinfectant at the farm level will reduce the number of viable organisms on the surface of the shell.

2. Hatchery

Objective: Reduction of the introduction of salmonellas and arizonas into the hatchery and of the transmission of salmonellas and arizonas at the time of hatching.

a. Hatching-egg sanitation

i. Fumigation with formaldehyde gas at the hatchery prior to setting or at time of setting.

b. Egg-dipping procedures

Such procedures are presently in a stage of experimental development for prevention of salmonella and arizona infection.

c. Hatchery sanitation

i. In addition to general hatchery sanitation, procedures should be taken to hatch eggs according to flock source to prevent cross infection during the hatching process. Each poult source should be continued to be kept separate during sexing, grading, detoeing, desnooding, and other hatchery procedures to reduce cross contamination.

ii. Individual hatcher exhaust ventilation systems will reduce cross contamination within the hatchery.

d. Hatchery monitoring program

A monitoring system for salmonellas and arizonas can be used to detect sources of contamination.

i. Infertiles and early dead embryos
ii. Fluff samples
iii. Cloacal squeezings at time of sexing
iv. Pipped, dead in the shell, cull poults
v. 10-day mortality brooder house
vi. Environmental sampling of litter and dust in brooder house at 10-14 days and 30 days

B. Environment of Breeder Farm and Market Flocks

Objective: Elimination of salmonellas and arizonas from the environment of the breeder flocks.

1. Confinement-type housing so constructed that it can be thoroughly cleaned and disinfected.
2. Control of free-flying birds.
3. Control of rodents and other pests (flies, snakes, beetles, etc.).

4. Control of visitors to the buildings.
5. Environmental sampling of litter and dust in growing houses during grow out period.

C. Feedstuffs

Objective: Elimination of salmonellas and arizonas in feedstuffs and complete feeds.

1. Encourage feed industry to develop procedures to provide salmonella-free complete feeds.
2. Regulatory agencies (USDA, FDA and State) provide guidelines to ensure elimination of salmonellas from feedstuffs and commercial vehicles used to transport feed ingredients and complete feeds.
3. Guidelines to feed manufacturers
   a. Follow a program of periodic testing of those feed ingredients that have a high risk of being contaminated with salmonella to obtain an indication of the effectiveness of your suppliers program to prevent salmonella contamination.
   b. Purchase feed and feed ingredients from reliable suppliers who adhere to appropriate good manufacturing and sanitation practices.
   c. Do not rely on sampling and negative test results alone as a means of salmonella control. Reliance should be placed upon the processing techniques and sanitation procedures of your suppliers as well as your own good housekeeping practices in the storage and handling of feeds to minimize the opportunity for salmonella contamination.
   d. Inspect incoming feed and feed ingredients for evidence of water damage and signs of fecal contamination by insects, birds, rodents, and other animals. Do not accept contaminated material.

For more details to supplement these recommendations refer to Miscellaneous Publication No. 739, The National Poultry and Turkey Improvement Plans and Auxiliary Provisions, USDA-ARS Subpart D #147.31-147.35, pages 15-16. May 1969.

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
FEDERAL CENTER BUILDING
HYATTSVILLE, MARYLAND 20782

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF
AGRICULTURE